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ERRATA

VOL. LXXVI

P. 276 and p. 283, curves of fig. 1 belong with legend of fig. 3, and vice versa

P. 366, lines 1 and 14, for gamete read spore

P. 366, line 13, for crossing over read separation

VOL. LXXVII

P. 228, legend below photograph, for Shutze read Schutze

P. 352, line 9 from bottom, after Nature insert **112**:

P. 352, line 3 from bottom, for heft 1 read heft 2

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THE

BOTANICAL GAZETTE

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FERN RUSTS OF ABIES

H. P. BELL

(WITH PLATES I–V AND TEN FIGURES)

Introduction

The fern rusts of *Abies* include most of the species of the genus *Uredinopsis*, and possibly at least one species of *Hyalopsora*. There are a number of other rusts that parasitize *Abies*, representing a number of distinct genera; but those of *Uredinopsis*, the main theme of the present research, are the most frequent and perhaps the foremost in interest and importance. This is certainly true in the case of *Abies balsamea.*

Many problems have presented themselves during the course of the investigations of this genus, but among them three types have proved to be especially attractive. First, there are many unsolved problems regarding structure, sporogenesis, and phylogeny. A second complex deals with the phenomena of heteroecism, biological strains, and hence the exact delimitation of species. A third group calls for recognition, namely, a determination of the amount and the effect of the injury caused to their hosts by the various species of *Uredinopsis*, for not infrequently they are responsible for extensive damage to the new foliage of balsam firs, particularly the younger trees, and one species, newly described, modifies the conformation of its host. It is to the first set of problems that most attention has been given, but the others have not altogether been overlooked.

While *Uredinopsis* is the principal subject matter of these studies, a limited place has been given to *Hyalopsora*, since infections of *Hyalopsora* on one of the fern hosts were so frequently encountered, in such close association with *Uredinopsis*, that they appeared to have a bearing on certain of the *Uredinopsis* problems. The results in this connection have exceeded expectations, for through the discovery of a second new *Peridermium* on *Abies*, and preliminary inoculation experiments, there is now good reason to believe that *Hyalopsora* should be listed among the fern rusts of balsam. If so, a new field of inquiry is opened up.

The materials for this research were obtained mainly from the Timagami Forest Reserve in the Province of Ontario, a forest area of upward of 6,000 square miles, lying about 300 miles north of Lake Ontario, and one that is richly covered with the balsam fir.

Taxonomy

Uredinopsis is one of the three genera of rusts known to parasitize ferns. It was founded by MAGNUS (18) in 1892 on the type species *U. filicina* (Niessl) Magnus. He studied this fungus (a form then known as *Protomyces? filicinus* Niessl) as it occurs on *Phegopteris vulgaris* Metten, and discovered that in addition to the so-called "stylospores," since shown to be urediniospores, there were hyaline spores imbedded in the leaf parenchyma. These he called endospores, and because of the existence of this additional spore form he placed the fungus in a new genus, which he called *Uredinopsis*, and he renamed this species *U. filicina* (Niessl) P. Magnus. In 1895 STÖRMER (23) observed and reported a second *Uredinopsis* on *Onoclea Struthiopteridis* (L.) Hoffm., which he found in Norway near Christiania, and named it *U. Struthiopteridis*.

It remained for DIETEL (6), however, to discover that the fungi placed in this new genus *Uredinopsis* were rusts. In 1895, while working on *U. Struthiopteridis* Störmer, he germinated the hyaline spores discovered by MAGNUS, and found that they gave rise to the typical four-celled promycelia of the Uredineae. Thus these spores were demonstrated to be teliospores, and the fungi producing them were accordingly classified as rusts. DIETEL also described the two known European species of *Uredinopsis*, *U. filicina* (Niessl)

Magnus and *U. Struthiopteridis* Störmer. He also described and named *U. Pteridis*, a rust on *Pteris aquilina*, which had been sent to him from California.

Up to this time the European mycologists were not aware that PECK had antedated them (1872) by describing a parasite on *Onoclea sensibilis* L. which later was recognized to be a *Uredinopsis*. PECK interpreted this fungus as one of the Fungi imperfecti, and named it *Septoria mirabilis*. In 1904, MAGNUS, after reviewing FARLOW and SEYMOUR's list (9), and a more recent publication by H. and P. SYDOW (24), in which they described a supposedly new form, *U. americana* (Sydow) on *Onoclea sensibilis*, re-named PECK's fungus *U. mirabilis* (Peck) P. Magnus, and interpreted *U. americana* Sydow as a synonym. He also reported that he had received from America, through Professor G. F. ATKINSON, specimens of two new species of *Uredinopsis*. One, on *Aspidium Thelypteris* (L.) Sw., he named *U. Atkinsonii;* the other, on *Osmunda cinnamomea* L., he named *U. Osmundae*.

About a month before the publication of the work by MAGNUS, SYDOW (25) had published the description of another species for America, *U. Copelandi* on *Athyrium cyclosorum* Rupr. Three years later, ARTHUR (2) published a complete list of the North American species of *Uredinopsis*, and included in it still another new species which he had found on *Phegopteris Dryopteris* (L.) Fee, and which he named *U. Phegopteridis*. In 1906 ARTHUR (1) had substituted for *Uredinopsis* the name *Milesia* of WHITE (29), but he immediately abandoned this name, for, as stated by GROVE (14), "the genus *Milesia* is now dropped, because it was founded on an imperfect state which might belong to any one of several genera." ARTHUR's list includes seven species: *U. mirabilis*, *U. Struthiopteridis*, *U. Osmunda*, *U. Atkinsonii*, *U. Phegopteridis*, *U. Pteridis*, and *U. Copelandi*. Of these, *U. Struthiopteridis* is found in both Europe and America, and the remainder in America only; *U. filicina* is apparently restricted to Europe.

The genus as a whole was so far described as occurring solely on ferns and possessing teliospores and two other spore forms. The main features in the descriptions can be summarized as follows. Teliospores, of one to four or more cells, were described as being scattered throughout the mesophyll. One of the other spore forms was always recognized as being thin-walled, colorless or white in mass, fusiform, beaked, and usually appendaged. This form was supposed to be borne singly on pedicels, and was observed and reported by all investigators. The third form was not reported for all species; it was illustrated as thick-walled, irregularly oval, and borne singly on pedicels. The identity of the teliospores had been proven by DIETEL (6). The fusiform spores germinated readily and immediately in a drop of water, and were recognized by all as urediniospores, but the function of the thick-walled spores was not understood. They had not been germinated except in one instance by DIETEL, who called them single-celled teliospores. MAGNUS considered them a second form of urediniospore; and ARTHUR, for lack of a better name, described them tentatively as aeciospores.

In 1913–14 considerable advance was made in the knowledge of the life history of *Uredinopsis* by FRASER (12, 13). He carried out inoculation experiments on the five species, which he had reported for Nova Scotia in 1910 (10), namely, *U. mirabilis*, *U. Struthiopteridis*, *U. Osmundae*, *U. Atkinsonii*, and *U. Phegopteridis*, and demonstrated that *Peridermium balsameum* Peck was the aecial stage of these rusts. This was the first announcement that *Uredinopsis* is characterized by heteroecism. In 1917 WEIR and HUBERT (27) made a further advance by connecting a peridermium on *Abies grandis* Lindl. and *A. lasiocarpa* Nutt. with *Uredinopsis Pteridis* on *Pteris aquilina* L. (*Pteridium aquilinum pubescens*).

Some confusion unfortunately has arisen regarding the identity of the *Peridermium* which WEIR and HUBERT referred to as *Uredinopsis Pteridis*. This is due apparently to lack of experimentation; they now regard the *Peridermium* as something new. JACKSON (15), however, claims that it is identical with *Peridermium pseudo-balsameum* (D. and H.) ARTHUR and KERN (3), and lists it as such. In absence of evidence to the contrary, I shall follow JACKSON'S example and refer to it as *Peridermium pseudo-balsameum*.

A further difference of opinion has arisen in regard to the identity of the previously described *Peridermium balsameum* and *P. pseudo-balsameum*. WEIR and HUBERT claim that they are the same, but it should be noted that there appear to be certain decided differences between the two. Thus, in *P. balsameum* the thickness of the aeciospore wall is 1–1.5 μ, and the pycnia are "in section hemispherical, 100–130 μ broad, 30–50 μ high"; whereas in *P. pseudo-balsameum* the thickness of the aeciospore wall is 2.5–3.5 μ, and the pycnia are "globose, large, 160–175 μ broad" (3). If, in addition to these differences, *P. pseudo-balsameum* attacks the second year needles, there should be no difficulty in distinguishing between these two, for in the east *P. balsameum* attacks the first year needles only. In this case there could not be much doubt that the *Peridermium* on the second year needles which WEIR and HUBERT associated with *Uredinopsis Pteridis* is really *P. pseudo-balsameum.*

There still remains the question of the life cycle of *Uredinopsis Copelandi.* On the basis of morphology only, WEIR and HUBERT consider that it should be regarded as a synonym of *U. Pteridis.* On the other hand, from observations made on the distribution of *U. Copelandi* in the field, JACKSON concludes that it should be connected with *Peridermium balsameum* on *Abies grandis* and *A. nobilis.* It is impossible to decide between these conflicting views until the life history of *U. Copelandi* has been worked out by means of inoculation experiments.

After reviewing the work of these investigators, the life histories of the species of *Uredinopsis* as demonstrated by inoculation can be summarized as follows. *U. mirabilis, U. Struthiopteridis, U. Osmundae, U. Atkinsonii,* and *U. Phegopteridis* have stages II and III on various species of ferns, but for each its own special set of hosts, and stages O and I (as *Peridermium balsameum*) on *Abies balsamea. U. Pteridis* has stages II and III on *Pteris aquilina* (*Peridermium aquilinum pubescens*), and stages O and I (as *Peridermium pseudo-balsameum*) on *Abies grandis, A. amabilis,* and *A. lasiocarpa. U. Copelandi* has stages II and III on *Athyrium cyclosorum,* but I have not been able to find any record of experimental work connecting this species with an aecial stage.

Reference should next be made to discussions among various students of the rusts regarding the individuality of the species of *Uredinopsis* as listed by ARTHUR. This subject involves the question of morphological species as contrasted with biological species, a distinction which has been overlooked in many of the conclusions reached. FRASER appears to be the only one who has approached this matter from an experimental point of view. In 1913 (13) he carried out cross inoculations with species of *U. mirabilis*, demonstrating that the spores of this species would infect its own fern host only, namely *Onoclea sensibilis*, and that other ferns remained free even after repeated attempts and most careful inoculation. He considered this sufficient evidence on which to establish *U. mirabilis* as a distinct species. His statement on the subject is as follows: "These experiments confirm the work of last year and indicate clearly that *Uredinopsis mirabilis* is a distinct species." This is as far as FRASER went. Another statement, to the effect that "the species of the genus *Uredinopsis* are not separated by any marked morphological differences" (13), has left him open to a misunderstanding on the part of some recent writers. Thus WEIR and HUBERT state: "FRASER in his last article came to the conclusion that all five species with which he had been working were identical with the exception of *U. mirabilis*." On reviewing FRASER's papers, I could not find any statement which could strictly be interpreted in that way. The latter quotation from FRASER appears to be his only pronouncement on the subject, and in that he certainly does not go as far as WEIR and HUBERT suggest.

The last two investigators have made an extensive comparison of the sizes of the spores of *Uredinopsis*. After examining the measurements they obtained, and reviewing the publications of ARTHUR and FRASER, they conclude that on morphological grounds all the species of *Uredinopsis* should be grouped in two divisions, namely, *U. mirabilis*, with *U. Struthiopteridis*, *U. Osmundae*, *U. Atkinsonii*, and *U. Phegopteridis* as synonyms; and *U. Pteridis* with *U. Copelandi* as a synonym. A different grouping of one of these species is suggested by JACKSON (15). After a comparison of morphological characters, he concludes that *U. Atkinsonii* is the same as *U. Copelandi*. The idea of combining species is carried to the extreme by RHOADS, HEDGCOCK, BETHEL, and HARTLEY (22), who record only one species of *Uredinopsis*, namely, *U. mirabilis*, and list all others named by ARTHUR as synonyms. They quote FRASER and WEIR and HUBERT in supposed support of this idea.

It appears to me to be still premature to recast the species of *Uredinopsis* as given by ARTHUR. There are questions which should first be solved, and also certain physiological and morphological differences that cannot be overlooked. For instance, the aecial stage of *U. Copelandi* has never been demonstrated by inoculation experiments, and yet such work is necessary before any statement can be made regarding the complete life history of this species. Also, WEIR and HUBERT point out that, as the aecial stage of *U. Pteridis* attacks the second year needles and not the young ones, the life history of this rust may be decidedly different from such a species as *U. mirabilis*, the aecial stage of which attacks young needles only. They suggest that the teliospores of *U. Pteridis* germinate in the late summer, and that the rust overwinters as mycelium in the *Abies* leaf. This may or may not be the correct explanation. In any case, the fact that the age of the needles attacked varies from species to species and yet is constant within each species, indicates the existence of a marked physiological difference. Of the forms which have come under my observation in the Timagami Forest Reserve, *U. Phegopteridis* occurs in great profusion early in the season, and lasts on the fern throughout most of the summer. *U. Atkinsonii* can also be found throughout the summer, but it occurs in small quantities only; *U. mirabilis* and *U. Osmundae* appear in large quantities during the last of July and August and mature rapidly. Of these two *U. Osmundae* is the more common. For the locality mentioned these conditions are constant. As for the morphology of these rusts, minor but constant variations are just as pronounced. The difference between the thicknesses of the aeciospore wall of *Peridermium balsameum* and *P. pseudo-balsameum* has already been noted. In *U. Osmundae* the layer-like character of teliospores is more highly developed than in any other species. This shows clearly in figs. 23 and 24. This feature of *U. Osmundae* has been noted by many investigators and is constant for the species. Other minor differences have been noted, such as extent of spore production, size of pustule, color of infected area, etc., and although these differences are very small, yet they appear to be constant. Thus the evidence would indicate that the number of species as given by ARTHUR should certainly not be reduced.

Spore forms of Uredinopsis

FRASER and WEIR and HUBERT in their experimental work demonstrated that all six of the species of *Uredinopsis* investigated are heteroecious, and that they produce all the ordinary rust spore forms, pycnospores, aeciospores, urediniospores, and teliospores. In addition to these, as already noted, MAGNUS called attention to single-celled thick-walled spores found in pustules along with the uredinia in certain species. From a histological point of view very little that is new has been observed with reference to the aeciospores; the greatest interest attached to them is in the times of their appearance for the different species, and in the ages of the needles on which they occur. On the other hand, the pycnia are of distinct interest, especially in a comparative study of the pycnia rust types found on the needles of *Abies*. An account of these is given with the description of new species in the latter part of this paper. An extended study has been made of the other spore forms, detailed accounts of which follow.

SINGLE-CELLED THICK-WALLED SPORES

Reference has already been made to the diversity of views held with regard to the single-celled thick-walled spores described by MAGNUS. They have been variously interpreted as resting urediniospores, teliospores, and aeciospores, but, so far as I can find, no record exists of any demonstration of their function by means of inoculation tests. As a matter of fact, neither their identity as *Uredinopsis* spores nor their function has ever been proved.

Unlike the appendaged fusiform urediniospore, this thick-walled form has not been reported for all species of *Uredinopsis*. Of the seven rusts of this genus listed by ARTHUR, the thick-walled spores are "unknown" in the case of three, *U. Osmundae*, *U. Pteridis*, and *U. Phegopteridis*. Also investigators have reported this spore form as missing even from examples of species where it was supposed to occur. For instance, FRASER (12), who must have collected and examined a large amount of material, states that "the spores, which have been regarded as aeciospores, were rarely present in the collections." In my own experience I have repeatedly hunted for these spores on *Onoclea sensibilis*, *Osmunda claytoniana*, and *Asplenium felix-foemina*, but without finding them in a single instance, although the ferns were heavily infected with their respective species of *Uredinopsis* and showed an abundance of fusiform urediniospores and teliospores. It is significant also that since the publication of the work of FRASER connecting *Uredinopsis* on the fern with an aecial stage on the balsam, the literature contains practically no mention of this thick-walled spore. The references to it are found chiefly in the earlier articles written by MAGNUS and DIETEL, and these investigators must have worked chiefly with dried material.

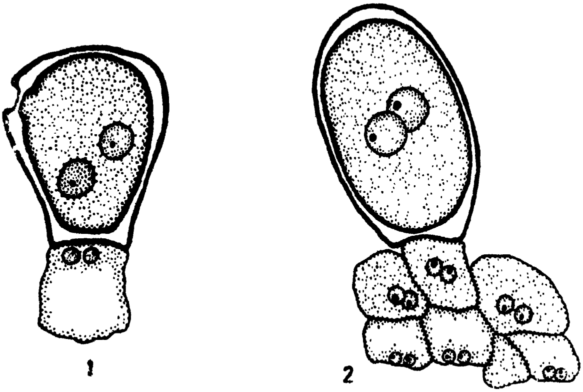
The persistence of DIETEL and MAGNUS in insisting on the existence of these spores as a phase in the life history of *Uredinopsis* is explainable in one of three ways: (1) that they do occur in certain species growing in certain localities, but not at all or irregularly in others; (2) that they are atypical urediniospores borne in young or suppressed pustules; and (3) that they really belong to some other species of rust simultaneously parasitizing the same host as the *Uredinopsis*. My experience would suggest that the third explanation is worthy of consideration.

I have observed thick-walled spores produced in great abundance on *Phegopteris Dryopteris*. In some cases small quantities of these spores were associated with rich productions of the fusiform urediniospore of *Uredinopsis Phegopteridis*. In such instances the thick-walled spores conformed to the spore type referred to *Uredinopsis*, and they were borne chiefly in unbroken pustules. At times they appeared to be coming out of the same pustule as the white filaments of the beaked fusiform urediniospores, but on close examination and sectioning, it was always found that the pustules of the two kinds were really separate. In the same district the *Phegopteris Dryopteris* was infected with *Hyalopsora Aspidiotus* (Peck) Magn. This was compared with *H. Polypodii-dryopteridis* Magn. (*H. Aspidiotus*) no. 2395 of the Sydow

Exsiccati. The two were found to be identical. This same *H. Aspidiotus* was then in turn compared with the thick-walled spores found with the *Uredinopsis*, and these two also were identical. On studying the distribution of the two spore forms in question, I found three conditions, as follows: (1) ferns infected with only the fusiform appendaged spores; (2) ferns infected with only the oval thick-walled spores; and (3) ferns infected with both spore forms. These three conditions often occurred close together, and also each condition was found by itself, and often some distance from patches of fern infected in either of the other ways.

To test this matter experimentally, on July 20, 1921, I obtained four boxes of *Phegopteris Dryopteris* plants, free from disease, with from two to four plants in each box. None of the ferns in the vicinity from which these ferns were collected was infected with *Uredinopsis* or *Hyalopsora* at that time or at any other time during the summer. I inoculated the ferns in two boxes with the beaked fusiform urediniospores, and kept the ferns in the other two boxes as controls. The spores used for these inoculations were taken from ferns carrying both kinds of spores. On July 29 pustules giving off white filaments of the beaked fusiform urediniospores had developed on the inoculated ferns; but pustules of the thick-walled spores did not develop either then or later. The controls remained free from any infection.

While examining and identifying the spores of *Hyalopsora* and the thick-walled spores found with the *Uredinopsis*, I was at first at a loss to know whether they were stalked or sessile. From the descriptions of these spores in the manuals, it appeared to be necessary to decide this point before making a satisfactory identification. In mounts made from scrapings of either *Hyalopsora* or the thick-walled type associated with the *Uredinopsis*, most of the spores had no stalk; but occasionally spores were observed with an apparent stalk attached. Also in hand sections most spores appeared to be sessile, but here and there a spore would appear to be borne on a pedicel. The literature on this subject is contradictory. ARTHUR (2) describes both the spores of *Hyalopsora* and the so-called thickwalled spores of *Uredinopsis* as being borne on pedicels. GROVE (14) describes those of *Hyalopsora* as sessile, and those referred to *Uredinopsis* as stalked. On the other hand, BARTHOLOMEW (4) has illustrated a sorus of *Hyalopsora* containing spores borne on fairly long stalks. My own studies on this feature were based on microtome and hand sections from carefully killed and fixed material. In the sections made they appear as illustrated in text figs. 1 and 2. The spore is the terminal cell of a row, and if these basal sterile cells come away with the spore, they look like a stalk. Of course, any increase in the spaces between these rows of basal cells invariably accentuates this stalklike appearance.



FIGS. 1, 2.—Urediniospores of *Hyalopsora Aspidiotus*, showing basal or stalklike cells; ×953.

Thus BARTHOLOMEW, who used a dissecting and teasing-out method, reports that the spores are always borne on pedicels. In the same way, the slightest deterioration in material (such as would be caused by a strong killing fluid), a slight delay between time of collecting and killing, and of course drying, causes these basal cells to collapse and assume more and more the appearance of specialized stalks, and it will be recalled that many of the original descriptions were made from dried plants. In most preparations, however, in whatever way made, these rows of cells form a compact mass at the base of the pustule, and the spores appear to be sessile. Thus although the thick-walled spores associated with *Uredinopsis*, in regard to their stalks, have been described differently from the urediniospores of *Hyalopsora*, I found them to be identical in this respect. Whether they should be considered as stalked or sessile remains an open question.

Still another difficulty arose in regard to the color of the thick-walled spore, and here also the descriptions in the manuals differ. Although these spores often have a distinct and characteristic shade, too much reliance cannot be placed on this point, especially in cases where the description states the color in terms which do not permit any variation, for I have found yellow and colorless thick-walled spores in the same pustule. Also, on certain material which was collected, pressed, and dried, the spores when fresh were a glistening golden yellow, but some months afterward, when thoroughly dried, most of the spores showed very little color, and many were absolutely colorless. In all cases observed the wall was always colorless. Thus again we find an instance in which the drying of the plants used in making the original descriptions, or perhaps a difference in the age or preparation of specimen, may account for the fact that these spores were sometimes described as yellow, and sometimes as colorless.

This evidence suggests that the thick-walled spores attributed to *Uredinopsis* in some instances at least have been really spores of *Hyalopsora;* and in cases where they were reported as being found with a *Uredinopsis* spore form, the host was really infected with two distinct organisms. Of course, infection work, especially with the reputed thick-walled spores of *Uredinopsis*, will be necessary to really prove this.

Before leaving this subject a brief comment should be made on two suggestions made by German investigators. First they laid great stress on the fact that the thick-walled spores are often borne in unbroken pustules, and on these grounds they conclude that the thick-walled spore might be a suppressed form of the beaked urediniospores. I have also observed a large number of unbroken pustules of these spores (fig. 21), and found them associated chiefly with a predominant growth of *Uredinopsis* and a rich production of the fusiform urediniospores of that genus. No difference could be detected, however, between the thick-walled spores in the unbroken pustules and those in the pustules which were broken open and discharging their spores, except that the spores in the unbroken pustules were often smaller and immature. Likewise, I do not find that there is any gradual transition between the thick-walled, oval spores and the thin-walled, beaked, fusiform urediniospores. I have examined unbroken pustules of each, and the many characters which clearly separate the mature spores can easily be detected in the young pustules, provided the proper technique is employed. Of course, each form varies so greatly that, if the young growths were examined on dried or poorly preserved material, the changes from the normal form might be such that the two kinds of spores and the pustules in which they are contained would appear to be very much alike.

Secondly, if, as MAGNUS suggested, this thick-walled spore is a second form of *Uredinopsis* urediniospore, one might expect the general characters of the two forms to be more alike than they are. For instance, the beaked fusiform spores are consistently hypophyllous, whereas the thick-walled oval spores are amphigenous. In fact, the latter are borne in a very irregular manner. Instances were observed in a number of different pustules where spores were borne on both sides of the mycelial mat. Fig. 27 shows this condition. In the section photographed only one spore shows on the inner side, but there were several in the other sections of the series through this pustule. In one striking case a spore had developed in the center of the mycelial mat at the base of the pustule (fig. 28). No such irregularity is found in the distribution of the beaked fusiform urediniospore.

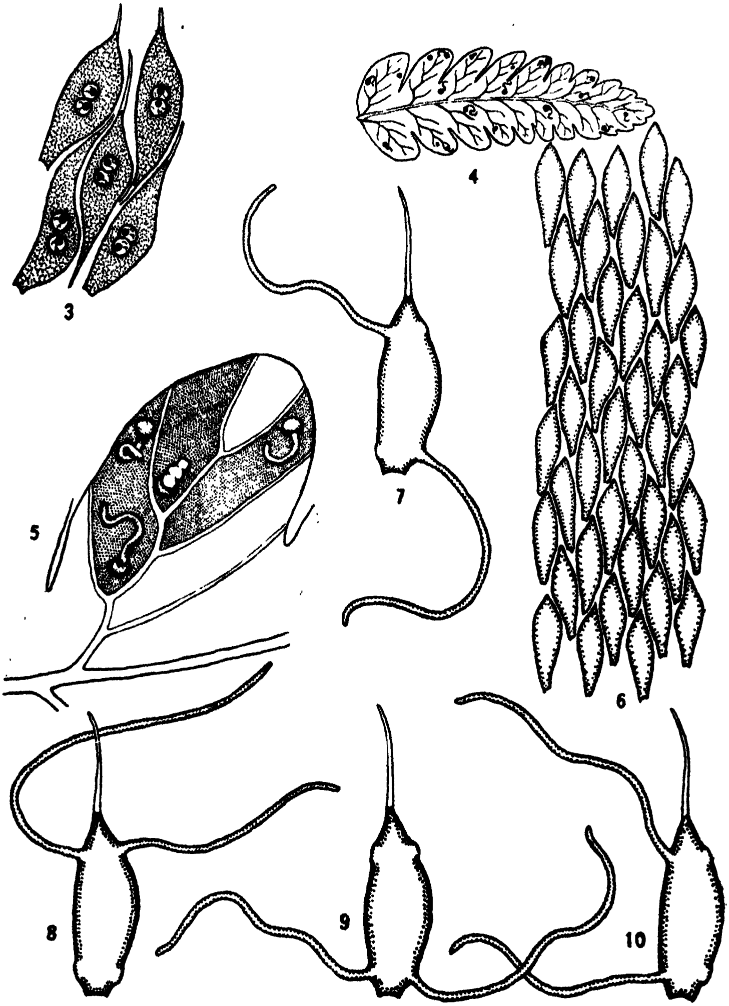
UREDINIOSPORES

The urediniospores of *Uredinopsis* are unique in form and in method of discharge. They are large, colorless, fusiform, single cells, usually appendaged, and, being slightly adhesive, emerge from the pustules in which they are produced in narrow ribbon or tendril-like masses, quite after the manner of the conidia of many of the imperfect fungi. The uredinial pustules are lined by a peridium consisting of binucleate cells, and the floor is made up of a mass of interlacing threads divided into binucleate cells; from this basal pad the binucleate spores originate. Hitherto the urediniospores have been supposed to arise singly and not catenulately, and on this ground the genus finds its place in the accepted systems of classification. In my studies of these spores special attention has been given to their mode of origin, supplementing by comparative studies on other suggestive forms; incidentally observations have been made on their markings and mode of germination. The only histological work which has been done on *Uredinopsis* is that of DIETEL (6) and MAGNUS (19, 20), apparently using hand sections and dried material only. They observed the peridium, but did not recognize the method by which the urediniospores were produced. They believed that these spores were borne singly on the floor of the uredinium, a conclusion not to be wondered at when the imperfections of the material used are taken into consideration.

METHOD OF PRODUCTION.—The species examined in my studies of urediniospore production are *U. mirabilis*, *U. Osmundae*, *U. Atkinsonii*, and *U. Phegopteridis*. The last named rust, which parasitizes *Phegopteris Dryopteris*, proved to be best adapted for this purpose. Figs. 1–15 and text figs. 3–10 were all drawn from specimens of that species. The material was collected in the Timagami Forest Reserve, Ontario, during the summers of 1919, 1921, and 1922. Some of it was killed in picro-sublimate and some in Flemming's weak chrom-acetic osmic solution. It was imbedded in paraffin, cut in sections 7 μ thick, and stained with iron alum haematoxylin, and either erythrosin or acid fuchsin as counter stains.

In collecting this material, I found it advisable to obtain it after a period of dry weather. The spores under such conditions escape from the sorus in a streamlike manner, often forming quite a long curved threadlike mass (text figs. 4, 5). This white thread or ribbon is seen through the microscope to be a mass of spores closely packed together; when flattened out slightly under the cover-glass, it appears as it is in part illustrated in text fig. 6. During a rain storm the spore tendrils and the mature spores in the pustules become dispersed, and a collection made at such a time may include many empty pustules.

In sections prepared in this way the development of an individual spore can usually be followed quite clearly. At first it is a roundish,



FIGS. 3–10.—Fig. 3, five immature urediniospores of *Uredinopsis*, illustrating peculiar shapes assumed during development; ×827. Fig. 4, pinna from frond of *Phegopteris Dryopteris*, illustrating uredinia and method of urediniospore discharge; ×2. Fig. 5, single pinnule of *P. Dryopteris*, showing uredinia and method of urediniospore discharge (infected leaf islets darkened); ×29. Fig. 6, portion of one of tendrils of discharging urediniospores as it appears under microscope when crushed out under cover glass; ×315. Fig. 7, germinating urediniospore of *Uredinopsis*, germ tubes coming from diagonally opposite pores; ×827. Fig. 8, germinating urediniospore of *Uredinopsis*, both germ tubes coming from pores at apical or distal end; ×827. Fig. 9, same, with both germ tubes coming from pores at basal or proximal end; ×827. Fig. 10, same, with both germ tubes coming from pores on one side of spore; ×827.

binucleate cell in the outer portion of the matted hyphae at the base of the sorus (figs. 1–3). This cell elongates and divides into two (figs. 4–6). The outer one elongates further and the inner one continues to divide. As these cells are cut off in this catenulate fashion, they at once begin to develop the fusiform shape of the mature spores. At the same time the connections between them become very narrow and delicate, and, as the spores mature, it is often impossible to distinguish the connecting walls. Figs. 8–11, showing the gradual development of the spore, were drawn from examples where the connection could still be distinguished. It is not usual to find more than three cells in an unbroken row. By this time the distal cell has nearly reached its complete development as a spore, and becomes detached by breaking the delicate wall connecting it with the cell immediately following (fig. 7). The remnant of the broken connection can be seen at the base of nearly all mature spores (figs. 12–15).

In a mature sorus so many separated spores become packed together that their catenulate origin would not be suspected except after a very close examination of the base of the pustule, and this is the stage at which the rust is usually collected (fig. 2). In a young sorus, when the spores are not fully developed, the catenulate formation is at once evident (fig. 1).

During the early stages of development, the spores appear to be very much crowded together, and often assume unusual shapes (text fig. 3). This crowding, and the narrowing of the connection between the cells, may play a part in the formation of the beak and appendage. That is, if the connection becomes drawn out and very narrow, and then breaks away from one spore, it may form an appendage for the other spore. This is also suggested by the fact that spores have often been observed with two appendages, one at each end. Others have an appendage at the proximal end only, and some have no appendage at all. As the break occurs most frequently as illustrated in fig. 7, however, the appendage may possibly not originate in this way, for it is not quite clear how the formation of a delicate appendage could result from such a wide and blunt connection. Although these irregular forms of spores are often seen, the most usual form is as illustrated in fig. 13. Such spores are appendaged on the distal end only, are more or less blunt, and show the remains of the original connection at the proximal end. Another very common form is illustrated in fig. 15, similar to fig. 13, except that the spore represented has no appendage, and often possesses a longer and thicker beak.

A knowledge of the catenulate mode of origin of the urediniospores of *Uredinopsis* is of importance, in that it throws light on the natural relationships of the genera of the Uredinaceae (Melampsoraceae). Most authorities (**2**, **8**) have grouped the genera of this family in the same general way. For instance, ARTHUR presents four sub-families, namely, the Uredinatae, the Pucciniastratae, the Chrysomyxatae, and the Cronartiatae. In only one of these, the Chrysomyxatae (containing the single genus *Melampsoropsis*), are the urediniospores described as catenulate; in the other three sub-families these spores are described as being "borne singly on pedicels." This classification as defined has already been shown to be inadequate, for investigations made on *Pucciniastrum Agrimoniae* by LUDWIG and REES (**17**) for *Pucciniastrum*, and investigations on *Melampsorella elatina* by MAGNUS (**20**) and LIRO (**16**) for *Melampsorella* have demonstrated the existence of catenulate urediniospores for these forms. While no further work has been published on other species of these genera, it is to be anticipated that they will show the same feature. These two genera were grouped by ARTHUR with *Uredinopsis*, *Hyalopsora*, and other genera in the sub-family Pucciniastratae. LUDWIG and REES pointed out that a readjustment was necessary, and on the basis of the data then at hand they suggested a new classification of this sub-family. They divide the Pucciniastratae into two groups, according to the existence or non-existence of catenulate urediniospores, and include all fern rusts in the non-catenulate group. As my investigations on the urediniospores of *Uredinopsis* indicate that they are catenulate, however, it is evident that their classification demands a second revision.

In order to have some check on my work on the urediniospores of *Uredinopsis*, I have examined the uredinia of *Pucciniastrum pustulatum* on *Epilobium angustifolium* and *E. adenocaulon;* and of *Melampsorella elatina* on *Stellaria graminea* and *Cerastium* *vulgatum.* Photographs of sections made from these are shown in figs. 30, 31, 29, and 32 respectively. As explained by LUDWIG and REES, very little can be learned from the arrangement of the mature spores in a pustule; it is only in an immature sorus that the fertile cells or potential spores retain their original positions. If the spores are given off in chains for any particular species, however, this phenomenon is indicated by the arrangement of the producing and developing cells at the base of even an old pustule. Decidedly young stages for any one of these four forms were not at my disposal, but the arrangement of the cells at the bases of their mature fructifications, and the stages in spore formation which were observable in the cells contiguous to the bases, certainly suggested that their urediniospores are produced in chains. On comparing the preparations made from these rusts with those made from the mature pustules of *Uredinopsis,* I found that the chained condition was even more apparent in *Uredinopsis* than in any one of the others.

MARKINGS.—Throughout this study I have had many opportunities of examining the wall of the urediniospores of *Uredinopsis* with reference to their surface markings. The wall of these spores has commonly been described as being characterized by "two opposite longitudinal thickened ridges bearing single rows of minute projections" (2). Occasionally I have observed a certain unevenness of their walls which exhibit the appearance as described, but in the great majority of cases the closest examination of material failed to reveal any such markings. The outside of the wall is seen best in perfectly fresh material examined in a dry mount; when wet the surface always appears to be smooth. In a suitable preparation the surface looks smooth for the main part, but here and there throughout are irregular minute projections, rough patches, and lines. Sometimes, as just stated, these surface markings appear to have a symmetrical arrangement and size, but usually they are irregular in every way.

GERMINATION.—The urediniospores of *Uredinopsis* germinate in a very characteristic manner, as has already been described by FRASER (11), as follows:

> Germ tubes emerged from germ pores, two placed near the beak and two near the base of the spore. The germ tube was that of the usual uredospore

but very small. Two germ tubes only emerged from each spore on germination, usually one from the oppositely placed pores either at the apex or base, but sometimes both on the same side of the spore.

These more usual methods of germination as described by FRASER are illustrated in text figs. 8–10, and text fig. 7 gives the outline of a germinating spore with the two tubes coming from diagonally opposite pores.

TELIOSPORES

The teliospores of *Uredinopsis Osmundae*, *U. mirabilis*, and *U. Phegopteridis* were collected and examined. The principal point of interest brought out was the discovery of their typical mode of distribution within the tissues of their host. The regular position for these spores was found to be just within the epidermis, and not "scattered through the mesophyll" as is usually stated. Occasionally some do occur in the intercellular spaces of the mesophyll, but these are few compared with the large number of spores which form a layer immediately under the epidermis, and most frequently under the lower epidermis. This is strikingly true of *U. Osmundae*, a feature which is shown in figs. 23 and 24, photographs of sections 7 μ in thickness. This layer-like grouping of the teliospores for *U. Osmundae* was observed and reported by MAGNUS (**19**). The same arrangement under the epidermis is illustrated for *U. mirabilis* in fig. 26, and for *U. Phegopteridis* in fig. 22. In both of the latter species they are more widely separated than in *U. Osmundae*, but in all three they are similarly disposed in a layer.

By the time the teliospores are mature the infected portions of the host are usually decidedly withered and not normal, as might be inferred from DIETEL's illustration (**6**, pl. 26, fig. 12). This withered condition is exhibited in figs. 22–26. Likewise the infected patches are yellow and are always limited by the fibrovascular bundles of the leaf (fig. 20); that is to say, the area of a lesion is that of a leaf islet. This limiting of an affected area is shown in fig. 25, in which the leaf veinlet located to the right of the center is seen to separate the withered shrunken tissue on the one side from the normal leaf tissue on the other.

New species

During the progress of this work two new species of *Peridermium* have been found on *Abies balsamea*, and a new form on *Polypodium vulgare*, all of them in the Timagami Forest Reserve. There is good reason to believe that all three are fern rusts of the balsam, and so an account of them properly finds a place in this paper. The last named is a species of *Uredinopsis* (*U. polypodophila*), the first to be reported for the common polypody. Its teliospores have not yet been observed, but the urediniospores were studied carefully, both from fresh material and thin stained microtome sections (fig. 42), and they are so characteristic that this fungus is unhesitatingly referred to the genus *Uredinopsis*. One of the peculiar features of this rust in comparison with other fern rusts is that it apparently fruits only on overwintered fronds; at all events, it has not been found on fronds of the current season.

Of the two species of *Peridermium*, one of them (*P. pycnogrande*) appears to be the alternate stage of the new *Uredinopsis* on *Polypodium vulgare*. Although this relationship has not been established by infection experiments, it is noteworthy that the two (the *Peridermium* on the balsam and the *Uredinopsis* on the common polypody) were always found together. This association was observed in many localities widely separated from one another. Likewise both hosts remained free from infection whenever they were found growing at some distance from one another.

This *Peridermium* is one of the most remarkable that attacks the balsam, not for its size or color, for in both of these features it resembles *P. balsameum*, but for the age of the needles on which it occurs, and its unusually large pycnia. It is always found on old needles, from two to eight years of age, whereas the other known species of *Peridermium* of the balsam, with the single exception of the one pointed out by WEIR and HUBERT (27) on the second year needles, are restricted to the new growth. The affected needles turn pale green, but are never blanched as those infected with *P. balsameum*.

The needles which bear the peridermia usually drop from the tree in the course of the following winter. This fact is indicated by the bared stretches of branch axes (figs. 17, 18) found contiguous to groups of infected needles. In order to establish this point, however, observations were made on certain living balsams in the Timagami Forest Reserve. Trees bearing branches which carried large, closely placed needles heavily infected with this *Peridermium* were chosen during the summer of 1921, and the limits of the infection were carefully marked. These marked branches were observed again in June 1922. By that time most of the needles which carried the infection during the summer of 1921 had dropped off, and later in the summer the needles adjacent to, but just outside the infection limits of the previous season, developed the typical white peridermia and large pycnia. An occasional needle which carried the infection of 1921 remained on the branch and developed peridermia again in 1922, in which case the blackened scars left by the 1921 peridermia could always be seen on the under surface of the leaf. Very commonly, too, the infected portions are associated with a peculiar multiplication of twigs, producing a loose broom effect (figs. 16, 19). These brooms are not found on a newly infected portion, but develop from year to year with the progress of the rust. This abnormal increase of branches never produces a dense mass of twigs, as in the case of *Melampsorella elatina*, but all the characters of a thicker broom are exhibited, although on a smaller scale. There is also a change in the normal direction of growth (figs. 16, 19). Fig. 19 shows the final loose broomlike effect.

The bark from infected branches was examined and fungus threads were found growing among the cells of the cortex. As yet, however, no work has been done to prove that the mycelium observed belonged to the same organism which produced the peridermia on the leaf, but the presence of fungus threads among the cells of the cortex, associated with the spread of the fungus from year to year up and down the branch, and the stimulation of the tree to abnormal growth, suggest that this *Peridermium* is perennial on *Abies balsamea*.

The second *Peridermium* (*P. pycnoconspicuum*) appears to be the alternate stage of *Hyalopsora Aspidiotus* (Peck) Magnus. The balsams affected with it were found constantly associated with *Phegopteris Dryopteris* bearing a copious growth of *H. Aspidiotus*. To test this relationship experimentally, preliminary tests were made as follows. On June 24, 1922, four boxes of *Phegopteris Dryopteris* free from disease, two plants in each box, were removed to the laboratory from a locality free from *Hyalopsora* and *Uredinopsis*, and which remained free for the rest of the summer. The ferns in three of the boxes were inoculated with the yellow aeciospores of this second new *Peridermium*, and were then left in a moist chamber for two days. They were then placed out of doors in a location far removed from other plants of *Phegopteris Dryopteris*, and at least two miles from any known infection of the *Hyalopsora*. The ferns in the fourth box were kept as controls. On July 12 pustules giving off the typical golden-yellow spores of *Hyalopsora* had developed on both plants in one of the boxes. A few days later one of the plants in a second box showed a similar infection; thus three out of the six inoculated ferns were infected with *H. Aspidiotus*, which may be considered a fair showing in view of the advanced age of the aecia from which the inoculum was taken, and some days of dry weather to which these delicate ferns were subjected. The controls remained free from infection. While these experiments cannot be considered as final, the results obtained from them and the phenomena of association in the field are strongly indicative of the suggested relationship.

There have been many speculations regarding the aecial stage of *Hyalopsora*. It has always been regarded as a peculiar genus, and its life history has been imperfectly understood and variously explained. Several investigators have assumed that the aecial stage of *Hyalopsora* might be found on a conifer, and some inoculation experiments have been made by them, although without success. BARTHOLOMEW (4), after studying this rust on the ferns, was convinced that its alternate stage would be found on conifers. Several years previous BUBÁK (5) had made cross inoculations on *Abies* and *Pinus*. DIETEL (7) in 1911 made further tests, and according to P. and H. SYDOW (26), BUBÁK and KLEBAHN inoculated, but without success, species of *Abies*, *Picea*, *Larix*, and *Pinus* with telia of *H. Aspidiotus*. WEIR and HUBERT (28) report that they made similar unsuccessful inoculations on species of *Abies*, *Tsuga*, and *Pteris* (*Pteridium*). Incidentally it may be noted that the last named investigators carried out tests that would indicate that the urediniospores of *H. Aspidiotus* and *H. Polypodii* may winter over. Further, in none of the studies on this genus has there been any report of inoculations in the other direction, that is, from a *Peridermium* to the fern host. Thus the experiment reported in this paper is the first evidence obtained by means of inoculation that *Hyalopsora* is a heteroecious rust, and that its aecial stage actually exists on a conifer.

This *Peridermium* has also proved to be interesting in comparison with the other species of *Peridermium* found on *Abies balsamea.* It is one of the earliest to appear. It is shorter than most others, and characterized by an early apical dehiscence of bright yellow spores. The affected needles are pale greenish and rather sparsely scattered. One of the most striking things observed was the fact that the rust appears to be restricted to the needles of the third previous year. Thus of twenty-nine specimens taken at random in June 1922, the rust was found in every instance on the needles of 1920 only, but perhaps the most important feature of all is the occurrence of conspicuous yellowish pycnia of very large size.

The discovery of such unusual pycnia lends added interest to a comparative study of the pycnia of the rusts found on the balsam. A complete survey of this subject would involve an examination of the pycnia of various species of *Pucciniastrum*, *Melampsora*, *Melampsorella*, *Hyalopsora*, and *Uredinopsis*. Incidentally it may be in place here to report that occasional pycnia were found associated with the peridermia of *Calyptospora columnaris;* but even the studies reported herewith indicate that the pycnia are very important diagnostic structures. To demonstrate this it will be sufficient to call attention to the pycnia of four species of *Peridermium* on *Abies balsamea*, namely, *P. balsameum* (*Uredinopsis* on the fern), *P. pycnogrande* (probably *U. polypodophila* on *Polypodium vulgare*), *P. pycnoconspicuum* (probably *Hyalopsora Aspidiotus* on *Phegopteris Dryopteris*), and *Pucciniastrum pustulatum* (telial stage on *Epilobium angustifolium*). Taking these in order, the distinguishing characters of their pycnia can be summarized as follows.

The pycnia of *Peridermium balsameum* (figs. 33, 34) are irregularly scattered; inconspicuous on the exterior; when mature extended both above and below the epidermis; in section hemispherical to spherical; comparatively small, 100–130 μ broad by 30–50 μ high.

The pycnia of *P. pycnogrande* (figs. 38, 39) are alternated regularly with the peridermia; inconspicuous on the exterior; subepidermal; deep seated in the mesophyll; in section conspicuous and spherical; large, 180–250 μ in diameter.

The pycnia of *P. pycnoconspicuum* (figs. 35–37) are alternated either regularly or irregularly with the peridermia, and sometimes in rows without any peridermia; conspicuous as round yellow spots on the exterior; extended well past the epidermis into the mesophyll; in section conspicuous and oval in outline; large, 400–500 μ long by 90–120 μ deep.

The pycnia of *Pucciniastrum pustulatum* (figs. 40, 41) are irregularly scattered; easily seen with a hand lens, but not conspicuous on the exterior; subcuticular and superficial, the epidermal cells being only partially occupied; in section flattened; small, 50–110 μ broad by 20–30 μ high.

The type specimens of the new species are deposited in the herbarium of the Department of Botany of the University of Toronto. The detailed description of them follows.

Peridermium pycnogrande, n. sp.—O. Pycnia hypophyllous, alternating with aecia in two rows, one on each side of midrib, forming distinct but not conspicuous light spots on the under surface. In section of leaf conspicuous, subepidermal, deep seated in the mesophyll, oblong or spherical, 180–250μ in diameter.

I. Aecia from mycelium freely distributed through mesophyll, hypophyllous, in two rows, one on each side of midrib, cylindrical, 0.25–0.3 mm. in diameter, and often 1 mm. high; peridermium colorless, delicate, rupturing at apex, becoming irregularly lacerate, cells elongated, 17–25×32–47 μ, walls thick, 2.5–3.4 μ, verrucose, overlapping; aeciospores globoid or broadly ellipsoid, 18–24×22–30 μ, wall colorless, rather thin, 0.8–1.3 μ, verrucose.

On *Abies balsamea* (L.) Mill. on two to eight-year old leaves, found near *Polypodium vulgare* around Lake Timagami, Ontario, Canada, appearing during July, August, and early September.

Peridermium pycnogrande, sp. nov.—O. Pycnidiis hypophyllis cum aecidiis alternis in duo series utrimque ad nervum dispositis

in maculis pallidis, distinctis sed non conspicuis, paginae inferioris; secto folio conspicuis, subepidermicis, penitus in mesophyllo insidentibus, oblongis v. sphaericis, 180–250 μ diam.

I. Aecidiis e mycelio orientibus copiose per mesophyllum distributo, hypophyllis, in duo series utrimque ad nervum dispositis, cylindratis, 0.25–0.3 mm. diam., saepe 1 mm. alt. Peridio hyalino delicato, apice erumpente, deinde irregulariter lacerato, cellulis elongatis, 17–25×32–47 μ, parietibus crassis, 2.5-3 μ, verrucosis imbruatis; aecidiosporis globoedis v. late ellipsoideis, 18–24×22–30 μ, tunica hyalina, subtenui, 0.8–1.5 μ, verrucosa.

Hab. in 2–8 annorum foliis *Abietis balsameae* (L.) Mill. prope *Polypodium vulgare* circum Lacum Timagami, Ontario, Canada, mensibus Julio, Augusto, et Septembri.

Uredinopsis polypodophila, n. sp.—II. Uredinia hypophyllous, few, minute, pale yellowish brown, 0.2–0.4 mm. across; peridium irregularly dehiscent, spores exuded in white filiform mass; urediniospores oval or fusiform, 45–55×15–23 μ, acute or acuminate above, with an apex often prolonged into a moderately strong beak 5–12 μ long; wall colorless, thin, 1 μ, smooth.

III. Not observed.

On *Polypodium vulgare* L., on fronds of the previous summer, found in shade of young balsam trees around Lake Timagami, Ontario, Canada, appearing during July, August, and early September.

Uredinopsis polypodophila, sp. nov.—II. Uredosoris hypophyllis, paucis, minutis, pallide flavobrunneis, 0.2–0.4 mm. diam., peridermio irregulariter dehiscente; sporis in molem albam filiformem exeuntibus; urediniosporis ovoideis vel fusiformibus, 45–55×15–23 μ, in super acutis vel acuminatis, apice saepe in rostrum moderate firmum 5–13 μ longum producto, tunica hyalina, tenui, 1 μ levi.

III. Teleutosporis ignotis.

Hab. in foliis anni praeteriti *Polypodii vulgaris* L. sub umbra arborum juniorum *Abietis balsamae*, circum Lacum Timagami, Ontario, Canada, mensibus Julio, Augusto, et Septembri.

Peridermium pycnoconspicuum, n. sp.—O. Pycnia hypophyllous, alternating irregularly with aecia in two rows, one on each side of midrib, forming conspicuous round yellow spots on the under

surface. In section of leaf conspicuous, oval, subepidermal, 400–500 μ long by 90–120 μ deep.

I. Aecia from mycelium freely distributed through mesophyll, hypophyllous in two rows, one on each side of midrib, bladder-like, spherical, low, 0.4–0.5 mm. in diameter and often only 0.2 mm. high; peridium colorless, delicate, rupturing at apex, cells oblong, 15–20×18–32 μ, walls thick, 2–3 μ; aeciospores globoid or broadly ellipsoid, 13–18×15–25 μ, yellow; wall colorless, thin, verrucose.

On *Abies balsamea* (L.) Mill. on leaves of the third previous season; that is, the collections of 1922 occurred on the needles of 1920. Found near *Phegopteris Dryopteris* affected with *Hyalopsora Aspidiotus* around Lake Timagami, Ontario, Canada, appearing during June.

Peridermium pycnoconspicuum, sp. nov.—O. Pycnidiis hypophyllis cum aecidiis in duas series irregulariter alternantibus utrimque ad nervum dispositis in maculis orbiculatis fulvis conspicuis paginae inferioris; secto folio conspicuis, ovatis subepidermicis, 400–500 μ long, 90×120 μ alt.

I. Aecidiis e mycelio copiose per mesophyllum distributo, hypophyllis in duas series utrimque ad nervum dispositis, vesicae similibus, sphericis, depressis, 0.4–0.5 mm. diam., et saepe 0.2 mm. modo alt; peridio hyalino, delicato, apice erumpente, cellulis oblongis, 15–20×18–32 μ, parietibus crassis, 2–3 μ; aeciosporis globoedis vel late ellipsoideis, 13–18×15–25 μ, clarissime; fulvis tunica hyalina, tenui, verrucosa.

Hab. in tertii anni antecedentis foliis *Abietis balsameae* (L.) Mill. i.e. anni 1922 collationes in foliis anni 1920 inventae sunt. Prope *Phegopterim Dryopterim Hyalopsora Aspidiota* affectis circum Lacum Timagami, Ontario, Canada, mense Junio.

Summary

1. *Uredinopsis*, originally defined as a genus of imperfect fungi parasitic on ferns, was proved to be a genus of rusts by Dietel in 1895. Heteroecism in *Uredinopsis* was first demonstrated by Fraser in 1913, when by means of inoculation experiments he established the relationship of some of the species to *Peridermium balsameum*.

2. The genus is characterized by four recognized types of spores: aeciospores, pycnospores, urediniospores, and teliospores. In

addition, some of the earlier investigators described a more or less irregularly occurring form on the fern host, interpreted variously as resting urediniospores, one-celled teliospores, and aeciospores. Studies recorded in this paper suggest that in some cases at least the urediniospores of a *Hyalopsora* have been misinterpreted as spores of the *Uredinopsis*. This type has never been found for any species studied, and a wide range of material was examined.

3 The urediniospores of *Uredinopsis*, unique because of their large size, fusiform shape, terminal appendages, and their emergence in spore horns, have heretofore been described as being borne singly. They are now shown to be produced in chains. Thus *Uredinopsis* should be grouped among those genera which are characterized by catenulate urediniospores.

4. No distinct evidence of constant markings on the urediniospores could be established.

5. FRASER'S account of the germination of the urediniospores is confirmed.

6. Teliospores of *Uredinopsis Osmundae*, *U. mirabilis*, and *U. Phegopteridis* have been studied carefully, and are found to occur in a more or a less regular layer immediately under the epidermis, and not irregularly scattered through the mesophyll, as commonly stated.

7. Three new species are described, namely, *Peridermium pycnogrande* and *P. pycnoconspicuum*, both on *Abies balsamea*, and *Uredinopsis polypodophila* on *Polypodium vulgare*.

8. Balsams affected with *Peridermium pycnogrande* were found commonly associated with the common polypody affected with *Uredinopsis polypodophila*, and field evidence indicates a connection between the two. The peridermia of this species are white throughout, and are accompanied by pycnia of very large size. This rust is also remarkable in that it occurs on needles from two to eight years of age and never on needles of the current season. This fungus is responsible for the growth of loose brooms in which it appears to be perennial.

9. Balsams affected with *Peridermium pycnoconspicuum* were found constantly associated with the oak fern *Phegopteris Dryopteris* affected with the uredinial stage of the rust *Hyalopsora Aspidiotus*

(Peck) Magnus. Field associations and preliminary culture experiments indicate that this peridermium is the aecial stage of *Hyalopsora Aspidiotus.* This is the first evidence indicating the heteroecism of any species of *Hyalopsora.* The peridermia of *Peridermium pycnoconspicuum* are columnar, the aeciospores are yellow, and the pycnia are large and conspicuous. The rust was found only on needles of the third previous season; that is, the collections of 1922 occurred on the still adhering needles of 1920.

10. The pycnia of *Peridermium balsameum, P. pycnogrande, P. pycnoconspicuum,* and *Pucciniastrum pustulatum* are here described and compared photographically; and they are shown to be distinctly characteristic for each species, and hence are important diagnostic features.

This work was done under the direction of Professor J. H. FAULL, University of Toronto, to whom I am indebted, not only for material and certain illustrations, but also for advice and assistance at every stage of this work. The investigations conducted during 1919 were made possible by a studentship awarded by the Honorary Advisory Council for Scientific and Industrial Research for Canada; and the summer work of 1921 and 1922 by a grant from the Special Research Fund of the University of Toronto. I wish to express my obligations to both the institutions from which this assistance was received. I am also under obligations to the Ontario Forestry Branch of the Department of Lands and Forests for the facilities extended in their laboratory in the Timagami Forest Reserve. For the translation of my English descriptions into Latin I am indebted to Miss MARY NEEDLER.

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EXPLANATION OF PLATES I-V

FIG. 1.—Camera lucida drawing of immature uredinium of *Uredinopsis Phegopteridis* on *Phegopteris Dryopteris;* ×953.

FIG. 2.—Composite drawing of mature uredinium of *Uredinopsis Phegopteridis* on *Phegopteris Dryopteris;* ×282.

FIGS. 3-11.—Stages in development of urediniospore of *Uredinopsis.*

FIG. 3.—Young spore in first stage of development amongst mycelium at base of sorus; ×827.

FIG. 4.—Spore as in fig. 3, after one division; ×827.

FIG. 5.—Later stage; there has been a second division in lower spore; cross wall is being formed; ×827.

FIG. 6.—Condition following that illustrated in fig. 5; small round cells starting to elongate; ×827.

FIG. 7.—Two cells of spore chain; distal cell is almost a mature spore (common form of development); ×827.

FIG. 8.—Protoplasm of two spores just starting to separate, leaving cell wall free; ×827.

FIGS. 9-11.—Spore chains illustrating more shrinkage of protoplasm than shown in fig. 8; ×827.

FIGS. 12-15.—Typical urediniospores of *Uredinopsis* all arranged with apex uppermost; fig. 13 illustrates commonest form; ×827.

FIG. 16.—Typical and fully developed broom on *Abies balsamea* infected with *Peridermium pycnogrande* (photographed by Dr. J. H. FAULL).

FIG. 17.—Branch of *Abies balsamea* infected with *Peridemium pycnogrande*; considerable defoliation taken place; most of needles dotted with white peridermia.

FIG. 18—Branch of *Abies balsamea* infected with *Peridermium pycnogrande;* peridermia show clearly; also loss of needles and abnormal development of twigs is just starting (photographed by Dr. J. H. FAULL).

FIG. 19.—*Abies balsamea* badly infected with *Peridermium pycnogrande*, and exhibiting abnormal multiplication of branches at various places (photographed by Dr. J. H. FAULL).

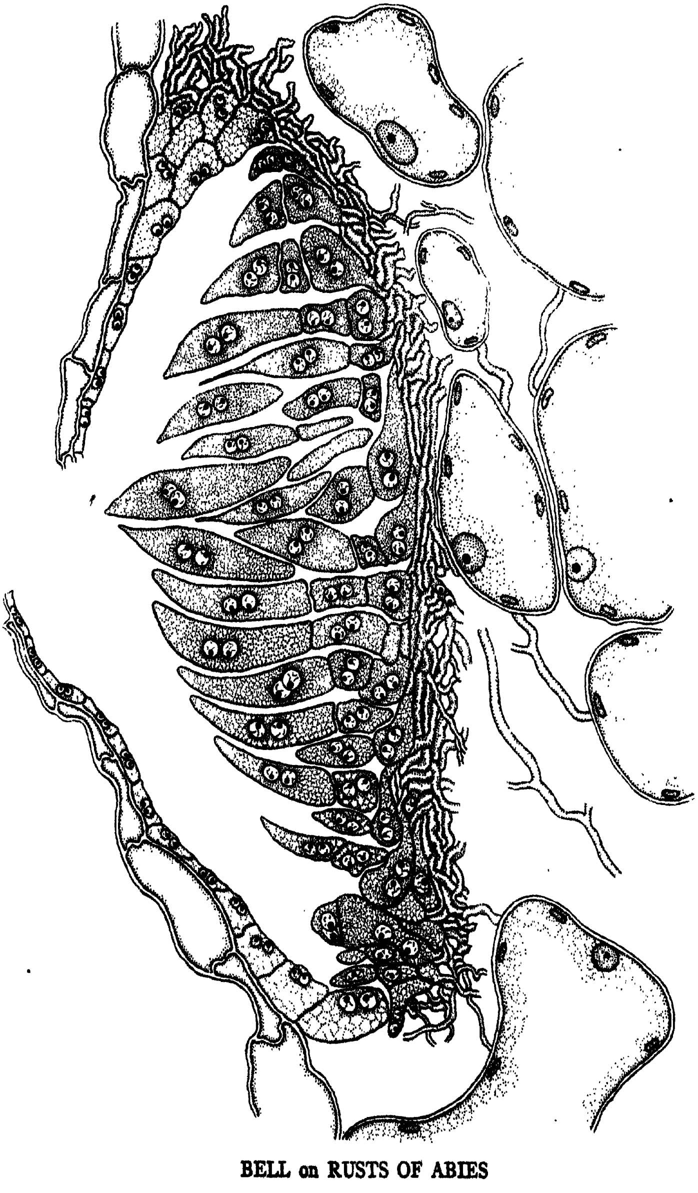
FIG. 20.—Pinna of *Osmunda Claytoniana* infected with *Uredinopsis Osmundae;* infected leaf islets are lighter color (photographed by Dr. J. H. FAULL).

FIG. 21.—Uredinium of *Hyalopsora Aspidiotus*, typical unbroken pustule so often found; break visible in covering layer of epidermis caused in cutting; ×200.

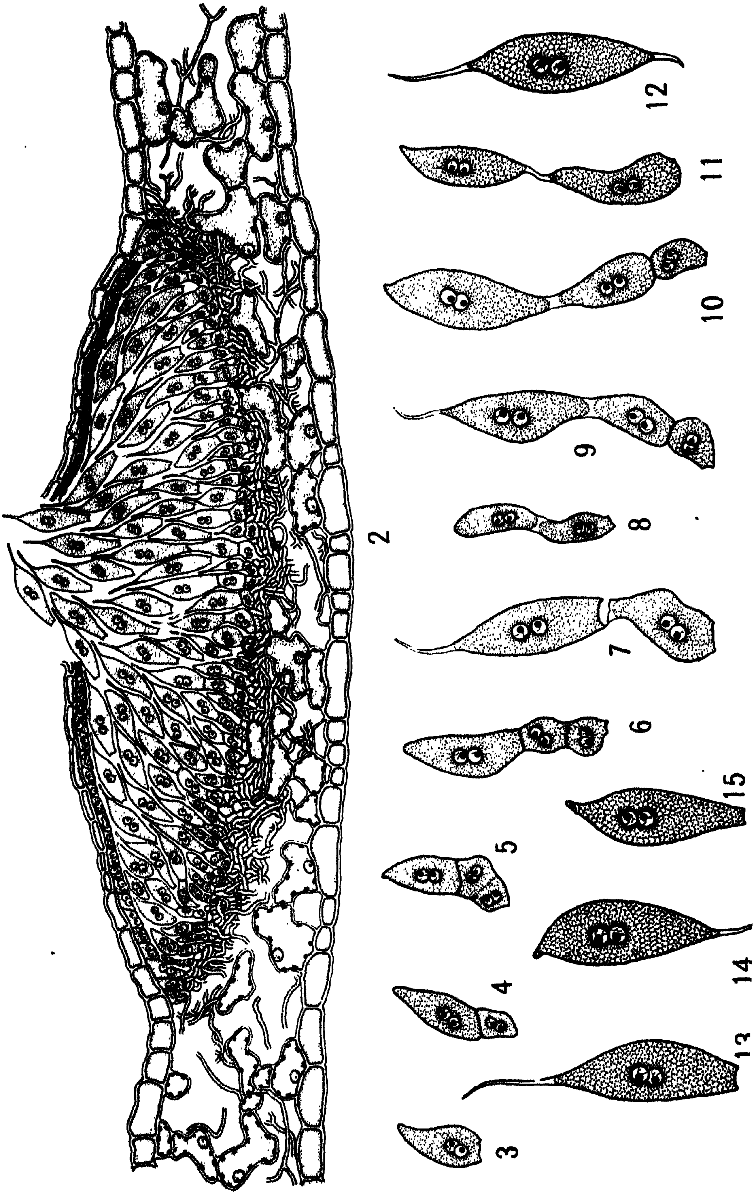
FIG. 22.—Teliospores of *Uredinopsis Phegopteridis* on *Phegopteris Dryopteris;* ×300.

FIG. 23.—Teliospores of *Uredinopsis Osmundae* on *Osmunda Claytoniana;* ×144.

FIG. 24.—Teliospores of *Uredinopsis Osmundae* on *Osmunda Claytoniana;* central part of section shown in fig. 36; ×300.



BELL on RUSTS OF ABIES



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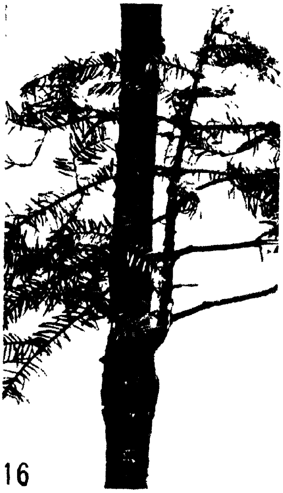
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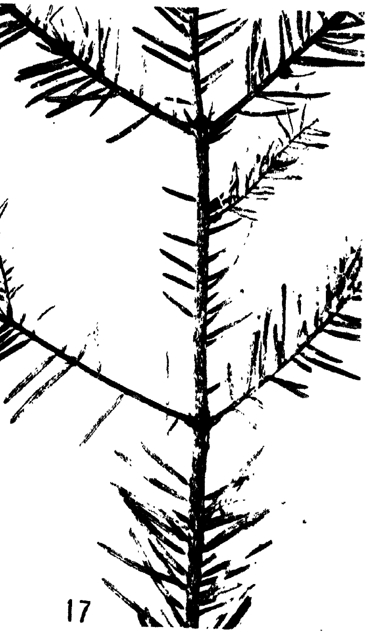
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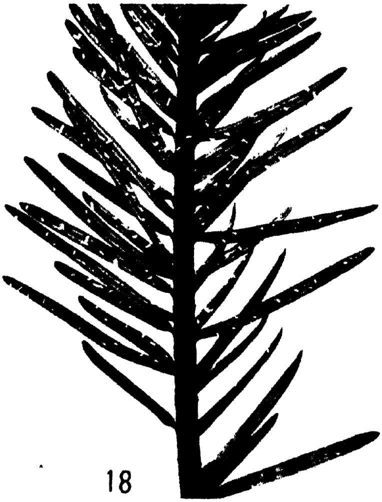
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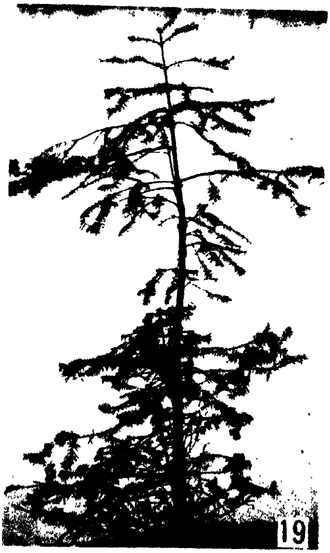
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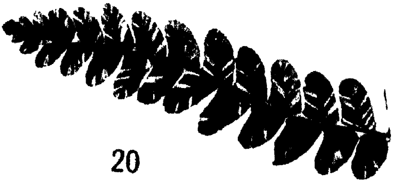
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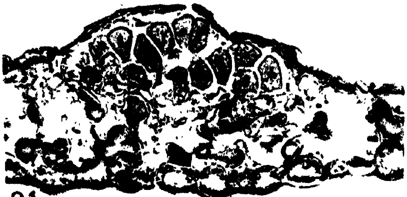
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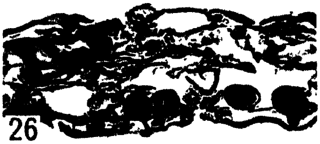
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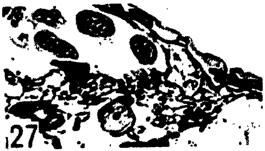
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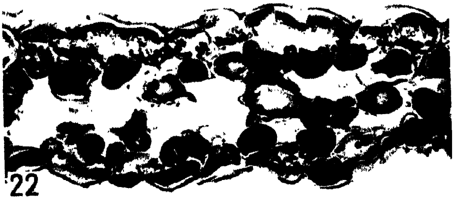
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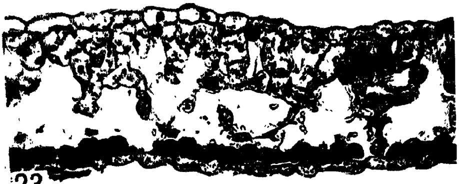
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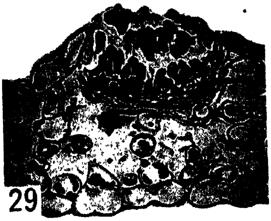
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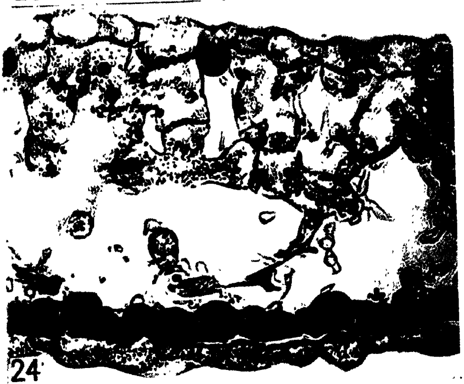
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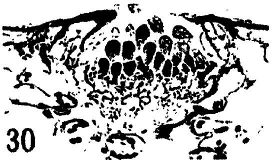
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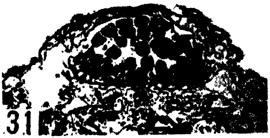
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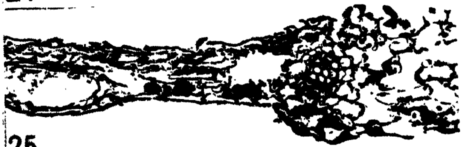
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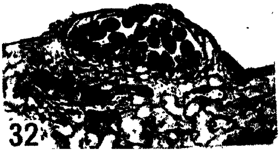
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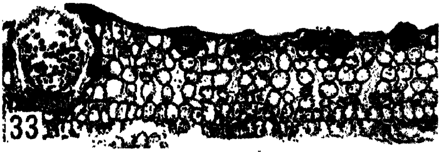
BELL on RUSTS OF ABIES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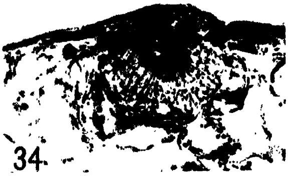
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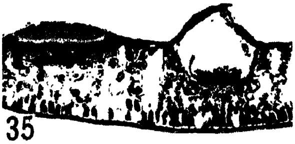
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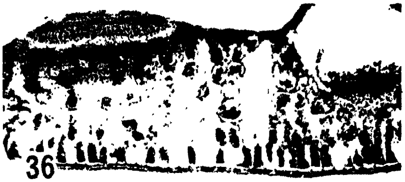
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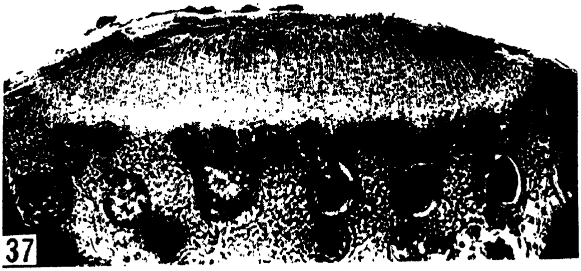
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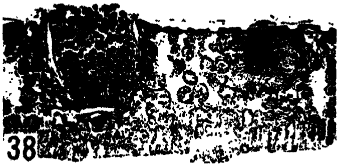
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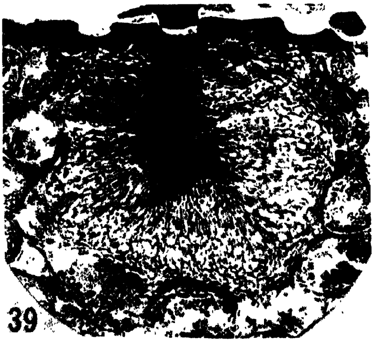
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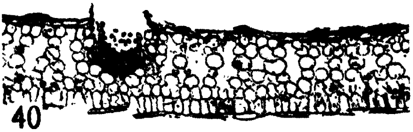
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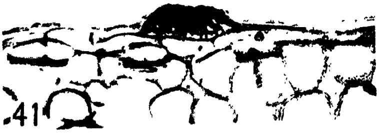
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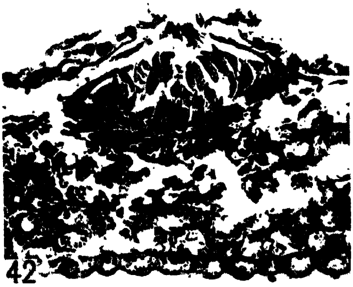
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BELL on RUSTS OF ABIES

FIG. 25.—Section of frond of *Onoclea sensibilis* infected with *Uredinopsis mirabilis;* infected and normal portions of leaf separated by fibrovascular bundle; infected portion withered and shrunken, showing four teliospores immediately under epidermis; ×144.

FIG. 26.—Teliospores of *Uredinopsis mirabilis* on *Onoclea sensibilis;* tissue of host decidedly withered; ×300.

FIG. 27.—Uredinium of *Hyalopsora Aspidiotus* (note that a spore is borne on inner surface of basal mycelial mat); ×200.

FIG. 28.—Uredinium of *Hyalopsora Aspidiotus:* at left hand end of pustule there is a spore borne in center of basal mycelial mat; ×200.

FIG. 29.—Uredinium of *Melampsorella elatina* on *Stellaria graminea;* ×200.

FIG. 30.—Uredinium of *Melampsorella elatina* on *Cerastium vulgatum;* ×200.

FIG. 31.—Uredinium of *Pucciniastrum pustulatum* on *Epilobium angustifolium;* ×200.

FIG. 32.—Uredinium of *Pucciniastrum pustulatum* on *Epilobium adenocaulon;* ×200.

FIG. 33.—Longitudinal section of leaf of *Abies balsamea*, showing aecium and pycnia of *Peridermium balsameum;* ×48.

FIG. 34.—Pycnium of *Peridermium balsameum;* ×200.

FIG. 35.—Longitudinal section of leaf of *Abies balsamea*, showing aecium and pycnium of *Peridermium pycnoconspicuum;* ×46.

FIG. 36.—Same as fig. 35; ×92.

FIG. 37.—Pycnium of *Peridermium pycnoconspicuum;* higher magnification of pycnium shown in fig. 36; ×200.

FIG. 38.—Longitudinal section of leaf of *Abies balsamea*, showing section and pycnium of *Peridermium pycnogrande;* ×46.

FIG. 39.—Pycnium of *Peridermium pycnogrande;* higher magnification of pycnium shown in fig. 51; ×200.

FIG. 40.—Longitudinal section of leaf of *Abies balsamea*, showing aecium and pycnia of *Pucciniastrum pustulatum;* ×46.

FIG. 41.—Pycnium of *Pucciniastrum pustulatum;* ×200.

FIG. 42.—Uredinium of *Uredinopsis polypodophila;* ×200.

NOTES ON NEOTROPICAL ANT-PLANTS

III. CORDIA NODOSA LAM.

I. W. BAILEY

(WITH PLATES VI, VII, AND FIVE FIGURES)

Introduction

There are numerous tropical plants that have curious nodal or internodal swellings which are inhabited by ants, such as species of *Myristica*, *Kibara*, *Anthobembix*, *Pleurothyrium*, *Humboldtia*, *Schotia*, *Platymiscium*, *Chisocheton*, *Aphanamixis*, *Endospermum*, *Barteria*, *Gertrudia*, *Maieta*, *Epitaberna*, *Cordia*, *Clerodendron*, *Nauclea*, *Uncaria*, *Randia*, *Plectronia*, *Cuviera*, *Sarcocephalus*, etc. Most of these myrmecodomatia are simple cauline hypertrophies, but certain of the neotropical species of *Cordia* have very complex structures, which are so remarkable morphologically as to justify discussing them at considerable length in the following pages.

Morphology

Two sections of the Borraginaceous genus *Cordia* are provided with myrmecodomatia, Physoclada and Gerascanthus. The most classical representative of these ant-plants is one of the Physocladae which is commonly referred to *Cordia nodosa*. SCHIMPER (16) encountered the plant in Pernambuco and studied its morphological peculiarities.

The large leaves which, like the stem, are provided with long reddish hairs, are alternate, paired or grouped in false verticils of four. The subnodal portion of the stem, subtending each verticil of leaves, is strongly thickened and angular, and usually (although not invariably) is provided with a long bladder-like swelling. This pouch is jacketed internally as well as externally by a cuticularized epidermis and numerous trichomes. It subtends the lowest of the four leaves, in the axil of which is a small apical outlet which is not excavated by the ants. Above this leaf the thickened and much compressed primary axis gives rise to lateral inflorescences which are inserted in the axils of the three remaining leaves.

SCHIMPER concluded that the myrmecodomatium is formed by the lateral enlargement of the base of the petiole of the lowest leaf, which is adnate to the compressed portion of the main axis. SCHUMANN (17) questioned the accuracy of SCHIMPER'S observations. He argued from analogies with supposedly similar domatia of *Duroia hirsuta* and of various Gerascanthi, that the inflorescences are terminal, and that the bladder-like swellings are cauline, rather than adnate foliar structures. MEZ (14) agreed with SCHUMANN that the inflorescences are terminal, but he inferred that the domatia are formed by lateral invaginations. His description of the morphology of the myrmecodomatia may be summarized as follows:

> The hypertrophied portion of the main axis bears two approximately opposite leaves, and terminates in an inflorescence. The lower leaf of this pair is subtended by the bladder-like swellings, and the axis of vegetative elongation, which is of secondary order, arises from its axil. The two remaining leaves of the false verticil are the first leaves of this secondary axis. Externally the bladder-like structure is delimited by two longitudinal grooves, which extend from the base of the myrmecodomatium to its apex. Serial transverse sections of the domatium reveal the following sequence of changes. Below the hypertrophy the stem is of normal structure. In passing upward through the transitional region, one of the grooves remains unmodified, whereas the other deepens, producing an embayment in the contour of the vascular cylinder and medulla. The pith splits, and extensions of the fibrovascular and cortical tissues invaginate or bend inward around the medullary cavity. The invagination continues until the cortical and fibrovascular tissues intersect and unite with homologous tissues on the opposite side of the stem. Thus the pith is divided and compressed into two narrow, curved strands, each of which is surrounded on all sides by fibrovascular and cortical tissues, and a commodious internal cavity is concomitantly formed which is jacketed by a cuticularized epidermis and numerous trichomes.

MEZ concluded that these invaginations were originated by minute insects which lived in the lateral grooves, and that they subsequently became accentuated and inherited as adaptations for housing ants. During the phylogenetic development of the myrmecodomatia, the margins of the longitudinal cleft, formed by the lateral invagination, fused together and isolated the internal layer of epidermal tissue. The position of this suture is indicated by a gap in the outer ring of sclerenchymatous tissue.

One of the commonest myrmecophytes of the Kartabo region of British Guiana is a *Cordia* which resembles the plant that SCHIMPER investigated in Pernambuco. Its dichotomously branching, hirsute cauline axes periodically form subnodal hypertrophies which subtend false verticils of four large hairy leaves and small inflorescences. These myrmecodomatia are jacketed both internally and externally by a cuticularized epidermis and numerous trichomes (except where they have been trimmed away by the ants), and are provided with preformed apical apertures. They are more or less elongate-turbinate, and differ from those described and figured by SCHIMPER in having no large, conspicuous, lateral, bladder-like swelling or pouch (text fig. 1). The four leaves of the false verticil are inserted at slightly different levels. The lowest leaf subtends a small axillary bud, which may occasionally develop into a lateral inflorescence. The leaf on the opposite side of the domatium is the highest of the four, and subtends the entrance aperture. The leaves of the remaining pair are inserted at right angles to this pair, and their axillary buds commonly give rise to dichotomous, lateral vegetative axes. Above the level of the leaves the main axis terminates in an inflorescence.

Serial transverse sections of the lower two-thirds of the myrmecodomatia show no indication of a putative flattened stem and adnate petiole, nor indeed of a lateral invagination (figs. 1, 4). The more or less circular wall is composed of concentric rings of highly differentiated tissues. Under higher magnification (figs. 5, 6) the sequence of tissues is seen to be: (1) outer epidermis, (2) cortical parenchyma, (3) sclerenchyma, (4) phloem, (5) xylem, (6) medullary parenchyma, (7) xylem, (8) phloem, (9) sclerenchyma, (10) cortical parenchyma, (11) inner epidermis. There are no connections between the rings of homologous tissue. The inner fibrovascular cylinder, whose tissues have an inverse orientation, is more or less rudimentary, and the increase in thickness of the wall of the domatium is due to cambial activity in the centrifugal or outer fibrovascular cylinder (cf. figs. 1 and 4). In passing upward toward the verticil of leaves, four arcs of the centrifugal cylinder become accentuated and project outward (fig. 9, text fig. 2). The longest arc of the four, which is a laterally enlarged basal projec-

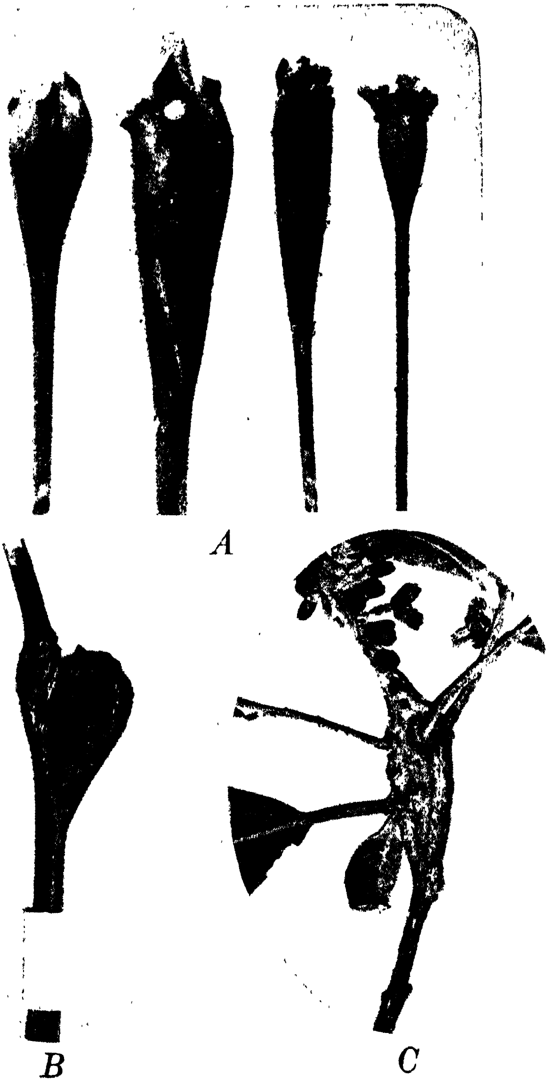

FIG. 1.—*A*, myrmecodomatia of *Cordia nodosa; B*, myrmecodomatium of *Cordia hispidissima* (Burchell no. 9637); *C*, myrmecodomatium of *Cordia Gerascanthus* Jacq. (Herbarium J. D. Smith no. 4365).

tion of the trace of the highest leaf, tends to split off from the fibrovascular cylinder before the others. As it does so, the centripetal fibrovascular cylinder divides into two portions, one of which becomes attached to the centrifugal cylinder and the other to the departing leaf trace. The former portion becomes an integral part of the centrifugal cylinder, whereas the latter portion gradually dies out at a higher level, leaving a normal arc-shaped trace which passes into the petiole of leaf (text fig. 3*A*). As indicated in this diagram, the entrance aperture is located in the axil of this leaf.

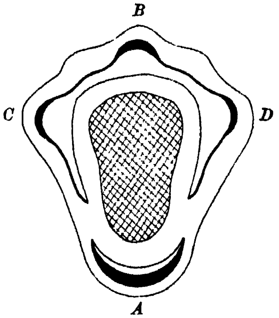

FIG. 2

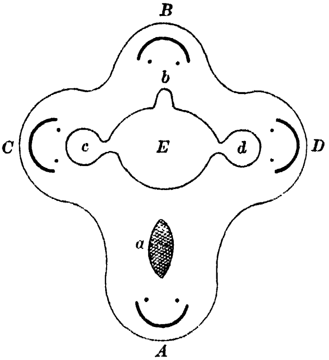

FIG. 3

FIGS. 2, 3.—Fig. 2, Transverse section of apical portion of myrmecodomatium of *Cordia nodosa*, showing traces of leaves *A*, *B*, *C*, and *D*.; fig. 3, Transverse section of apical portion of myrmecodomatium of *Cordia nodosa*, cut at somewhat higher level than that shown in fig. 2: *a*, entrance aperture in axil of leaf (*A*); *c*, *d*, axes of vegetative shoots in axils of leaves (*C*) and (*D*); *b*, rudimentary bud in axil of leaf (*B*); *E*, axis of terminal inflorescence.

The opposite leaf (*B*) subtends a dormant bud (*b*). Leaves *C* and *D* subtend the lateral vegetative shoots *c* and *d*, and the main axis (*F*) terminates in an inflorescence.

What then is the morphological significance of these extraordinary structures? The hypotheses of SCHIMPER, SCHUMANN, and MEZ do not afford an adequate explanation of all phases of their ontogenetic or phylogenetic development. Below the subnodal hypertrophy, the stem is of normal structure. Above this level it rapidly increases in girth. As it does so, the circumference of its concentric layers of epidermal, cortical, and fibrovascular tissues becomes correspondingly enlarged, and a commodious internal cavity is concomitantly formed in the dilated core of medullary parenchyma. This chamber, unlike that of the caulinary domatium of other myrmecophytes, is characterized by being jacketed by centripetal layers of epidermal, cortical, and more or less rudimentary fibrovascular tissues. In other words, there is no indication of a compressed stem and adnate petiolar enlargement, nor of an extensive lateral invagination. The centripetal layers of epidermal, cortical, and fibrovascular tissues unite with the homologous centrifugal layers only in the apical portion of the wall of the domatium which surrounds the small circular aperture.

A detailed study of the morphology of the myrmecodomatia, during successive stages of their ontogeny, indicates that they are hypertrophied portions of the cauline axes, whose medullary cavities are jacketed by layers of invaginating tissues. The invagination does not originate, however, in a longitudinal lateral groove, but in the axil of one of the leaves of the pseudo-apical verticil. As it develops, it produces an elongate-saccate ingrowth of the epidermal, cortical, and fibrovascular tissues into the rapidly enlarging core of medullary parenchyma (text fig. 4). The absence of even a rudimentary bud in the axil of a leaf which subtends an entrance aperture suggests that this growing point may be concerned in the formation of the invagination. If it is, the invagination may be visualized as the homologue of an ingrowing lateral shoot, and its formation may be likened to what happens when one finger of a glove is retracted so that it ultimately projects inward instead of

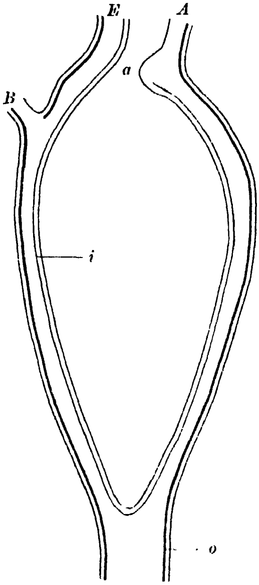

FIG. 4.—Longitudinal section of myrmecodomatium of *Cordia nodosa*, showing centrifugal (*o*) and centripetal (*i*) fibrovascular tissues: *a*, entrance aperture in axil of leaf (*A*); *E*, terminal inflorescence.

outward. In exceptional cases, invaginations may develop in the axils of two of the leaves of a single verticil. Fig. 8 illustrates a transverse section of an abnormal, compound domatium of this type.

It is of interest to compare these myrmecodomatia with those of other representatives of the Physocladae which have conspicuous, lateral, bladder-like swellings. Burchell's no. 9637, which is referred to *Cordia hispidissima* DC., and the Rusby Expedition's nos. 778 and 1035 have myrmecodomatia of this type. Serial transverse sections of these hypertrophies reveal the following histological details. Below the domatium the stem is of normal structure, but above this level it becomes constricted by two longitudinal furrows, and the pith appears bilobed. An internal cavity is formed in one of these lobes, which is jacketed by layers of inversely oriented epidermal, cortical, and fibrovascular tissues (fig. 7). The arc of the outer or centrifugal fibrovascular cylinder which surrounds this lobe becomes detached. As it does so, the inner or centripetal fibrovascular ring splits into two arcs, each of which attaches itself to one of the two outer arcs of homologous tissue (fig. 2). As the domatium enlarges, one lobe-shaped portion of the axis, the one which partially surrounds the internal cavity, enlarges more rapidly than the other and forms the bladder-like, lateral hypertrophy (cf. figs. 2 and 7). In the apical portion of the domatium, this lobe contracts, the inner arc of fibrovascular tissue fades away, and the outer arc passes into the petiole of the leaf which subtends the entrance aperture. The outer arc of fibrovascular tissue in the opposite lobe gives off three leaf traces, and in combination with the inner arc, subsequently forms the steles of four cauline axes, three lateral and one terminal (text fig. 3). The terminal axis forms the inflorescence. One or more of the lateral axes elongate to form vegetative shoots, or in certain cases lateral inflorescences.

It is evident that the myrmecodomatia of these plants are of the same fundamental morphological type as those which the writer studied in British Guiana. The striking difference in their external form is due to the fact that in the case of the former plants the subnodal enlargement of the stem is unilateral, and the basal projection of the trace of the leaf which subtends the entrance aperture is set off from the central cylinder at a much lower level.

Such structural differences are of not uncommon occurrence in closely related species of dicotyledons. Thus the basal projections of the leaf traces in one species or variety may be differentiated or even set off from the stele at a much lower level than they are in others. In view of these facts, the following conclusions concerning the morphological peculiarities of the Physocladae seem justified: (1) the myrmecodomatium is formed by an invagination of epidermal, cortical, and fibrovascular tissues which originates in the axil of one of the leaves of the false verticil, and which develops into the interior of a more or less symmetrical or unilateral, subnodal enlargement of the cauline axis; (2) this cauline axis usually terminates in an inflorescence; (3) the buds in the axils of three of the leaves of the false verticil of four give rise to one or more lateral axes of vegetative elongation, but in certain cases may form one or more lateral inflorescences.

The next question is, are the myrmecodomatia of the Gerascanthi homologous morphological structures? Although the writer has not encountered these myrmecophytes in the field, he has studied dried specimens of their myrmecodomatia in the extensive collections of the National Herbarium, the New York Botanical Garden, and the Gray Herbarium. The domatia vary greatly in size, shape, and distribution in different representatives of even a single species. When present, they are irregularly shaped hypertrophies of the axis of the large diffuse inflorescences or of the transitional region between cauline and floral axes (text fig. 1). The lateral entrances or exits are not preformed apertures, as in the Physocladae, but evidently are excavated by insects. Furthermore, the domatia are not jacketed internally by invaginated layers of epidermal, cortical, and fibrovascular tissues. As shown in fig. 3, a transverse section of a myrmecodomatium of *Cordia Gerascanthus* Jacq. (Herbarium J. D. Smith no. 4365), the inflated central cylinder surrounds a large heterogeneous medulla, the large celled, succulent core of which has dried up and has been trimmed away by the ants. In other words, these structures are quite distinct morphologically from the myrmecodomatia of the Physocladae, and resemble those of the Ethiopian species of *Cuviera* and *Plectronia* (BAILEY 3).

Taxonomy

Many of the discrepancies in the conclusions of various investigators who have concerned themselves with the phenomenon of myrmecophytism in *Cordia* are due to excessive generalization from limited induction. Thus, in general, SCHUMANN'S conclusions are applicable to the Gerascanthi, but not to the Physocladae. Furthermore, there has been a tendency to base critical morphological and ecological observations upon plants which have not been identified with certainty, and of which no herbarium specimens are available for verification. This naturally has led to considerable confusion.

As previously stated, SCHIMPER believed that his myrmecophyte was *Cordia nodosa*, but SCHUMANN and MEZ questioned his determination and considered the plant which had hirsute stems and leaves to have been *C. hispidissima*. SCHUMANN suggested, however, that the latter species may be a variety of *C. nodosa*. Unfortunately the taxonomy of the Physocladae is a somewhat complicated problem. AUBLET (1) described and figured a *Cordia* which he referred to *C. Collococca*. LAMARCK (13) subsequently gave to this plant the specific name *C. nodosa*. DE CANDOLLE (9) distinguished two additional species of Physocladae, *C. miranda* and *C. hispidissima*, which are characterized by having compact, sessile inflorescences. FRESENIUS (12) retained these two species, and described three forms or varieties of *Cordia nodosa:* (1) *glabrior*, based on *C. formicarum* Hoffmsg.; (2) *hispidissima*, based on AUBLET'S plant; and (3) *angustifolia*. It is evident from AUBLET'S detailed descriptions and figures that the plant which LAMARCK referred to *C. nodosa*, and FRESENIUS to var. *hispidissima*, possessed densely hirsute stems and leaves, and small, but diffusely and dichotomously branching pedunculate inflorescences. The stems, leaves, inflorescences, and fruits of the myrmecophytic *Cordia* of the Kartabo region of British Guiana closely resemble those of this plant. In view of the fact, therefore, that AUBLET'S and the writer's plants were collected in the same general region (French Guiana and British Guiana), it is reasonable to assume that they both belong to the same species.[1]

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[1] The herbarium specimens collected by the writer, nos. 39, 40, 41, and 42, are deposited in the Gray Herbarium of Harvard University.

The myrmecodomatia of AUBLET'S plant, of the writer's myrmecophyte, of the specimen of *C. nodosa* figured by FRESENIUS, and of two somewhat similar but subglabrous plants collected by J. A. SAMUELS in Dutch Guiana appear to be devoid of large, conspicuous, lateral, bladder-like swellings. On the contrary, the domatia of Brazilian and Bolivian Physocladae (Spruce no. 3281, Burchell no. 9637, Rusby Expedition nos. 778 and 1035, and SCHIMPER'S plant) are characterized by having these unilateral, pouchlike enlargements. In view of the fact that certain systematists are inclined to group the various Physocladae together as varieties of a single species, upon the assumption that the shape and texture of the leaf, pilosity, and degree of compactness of the inflorescences are exceedingly variable, the morphology of the myrmecodomatia may prove to be of some diagnostic value. In the case of the Kartabo myrmecophyte, the form and structure of the myrmecodomatia are very constant. The writer collected material from a wide range of plants of different ages, sizes, and localities. All of the domatia were devoid of conspicuous, lateral, bladder-like enlargements. It will be of interest to determine whether these structures are equally constant in plants of other neotropical regions.

Ecology

Most of the earlier students of ant-plants considered that the structural peculiarities of the plants are originated directly by the ants. SPRUCE (20) and BECCARI (6) were forced to admit, however, that many of these abnormalities (ant-galls) have become inherited. That the myrmecodomatia of certain groups of ant-plants are produced by gall-insects rather than by ants, has been suggested from time to time by various investigators. CHODAT and CARISSO (10) have even formulated "*Une nouvelle théorie de la myrmécophilie*" upon the basis of the fact that they found eggs and larvae of hymenopterous parasites in immature myrmecodomatia of *Cordia glabrata*, *C. longituba*, *C. alliodora*, *C. Gerascanthus*, *C. Gerascanthoides*, and *Acacia Cavenia*. The title of their short paper might lead one to suppose that the formicaries of all myrmecophytes originate as galls. As a matter of fact, they qualify their generalization as follows: "Il y a tout lieu de penser que les formicairès des autres plantes myrmécophiles, quand ils se présentent comme des renflements, ont une origine analogue."

It is to be emphasized in this connection that the mere occurrence of ants, or even of gall-insects, in hypertrophied portions of the stem or leaf does not indicate necessarily that these structures are of traumatic origin. They may be inherited peculiarities which serve as convenient nesting chambers for ants, or, during the earlier stages of their ontogeny, as suitable places for the oviposition of other insects. The large stipular thorns of many of the Mexican, Central American, and West Indian bull's-horn Acacias are not abnormalities produced by insect parasites, as evidenced by the fact that they are formed on plants which are not subject to the visitation of ants or of gall-insects. The ants are able to nest in the thorns regardless of whether they have previously been occupied by gall-insects or not. It is doubtful whether they are able to do so in the case of certain of the East African species of *Acacia*. The stipular thorns of these plants are straight and rather slender, but may be deformed at times by huge basal hypertrophies. SCHWEINFURTH (18), SJÖSTEDT (19), SCHENCK (15), and others are of the opinion that these apparent abnormalities actually are true galls. Although the observations of these investigators are not entirely conclusive and need to be verified by further and more detailed field studies, or by controlled experiments with plants grown from seed, it seems probable that ants are unable to utilize these thorns as formicaries unless they have previously been enlarged by gall-insects.

The myrmecodomatia of the Kartabo *Cordia*, during the earlier stages of their ontogeny, do not contain insects or parasitic fungi, and there is no histological or ecological evidence to indicate that they are originated by ants or by gall-insects. As in the case of the Ethiopian myrmecophytes, investigated by BEQUAERT (8) and the writer (3), and in that of *Tachigalia paniculata* discussed in the second paper of this series (5), the myrmecodomatia are inherited structures which are taken possession of by ants after their tissues have become highly differentiated. The subnodal enlargements of the Physocladae, like the stipular thorns of Mexican and Central American bull's-horn Acacias, evidently serve as formicaries without previously being modified by gall-insects.

It would not be safe to infer from this, however, that the enlargements of the fertile or vegetative axes of the Gerascanthi are formed without the intervention of parasitic insects. The irregular shape and more or less sporadic distribution of the cauline hypertrophies in this section of *Cordia* suggest that, if the myrmecodomatia are not actually originated by gall-insects, they may be considerably accentuated by them. Extensive and critical field observations are needed to determine whether the immature hypertrophies always contain eggs or larvae of parasitic insects, and if so whether homologous structures are formed on plants grown from seed, which are protected from the activities of ants and of gall-forming parasites.

Belt (7), Delpino (11), and Schimper (16) formulated the hypothesis that the structural peculiarities of ant-plants originated through the action of natural selection, as adaptations for attracting ants which protect their hosts against the attacks of phytophagous insects, particularly of the destructive leaf-cutting ants. That the guest-ants of *Cordia nodosa* in the Kartabo region of British Guiana do not afford an efficient protection against *Atta cephalotes* is illustrated in text fig. 5. Although the myrmecodomatia of this plant contained flourishing colonies of *Allomerus 8-articulatus*, many of its leaves were cut into pieces and transported to the subterranean fungus gardens of the Attine ants.

My colleague, Professor W. M. Wheeler, has identified the following ants, taken from myrmecodomatia of the Kartabo *Cordia: Allomerus 8-articulatus* Mayr, *Azteca ulei* Forel var. *cordiae* Forel, *A. instabilis* F. Smith, *A. trigona* Emery subsp. *mediops* Forel, *Neoponera unidentata* Mayr, and *Crematogaster limata* F. Smith subsp. *ludio* Forel.

The first two species are "obligatory" guests of *Cordia nodosa* or closely allied Physocladae, that is, they are known only from these plants. The remaining ants are "facultative" or more or less ubiquitous species which nest in various habitats. *Allomerus 8-articulatus* is one of the commonest guest-ants of the Kartabo *Cordia.* It is characterized by building earthern galleries which extend from the entrances of the domatia down the outer surfaces of the branches and stem, to the level of the ground.

Although all of these ants have the general formicine habit of gnawing upon the walls of their domatia, they do not excavate the deeply sunken, pitlike or groovelike depressions which are such characteristic features of the myrmecodomatia of *Cecropia*, of *Tachigalia*, and of many of the Ethiopian ant-plants. Their failure to do so is not due to an absence of coccids, since the latter insects usually are present in domatia which contain well established and flourishing colonies of ants. It may be significant, in this connection, that the mature myrmecodomatia of *Cecropia*, *Cuviera*, *Plectronia*, *Nauclea*, etc., are jacketed internally by compact layers of very thick walled cells, and that the coccids feed only in

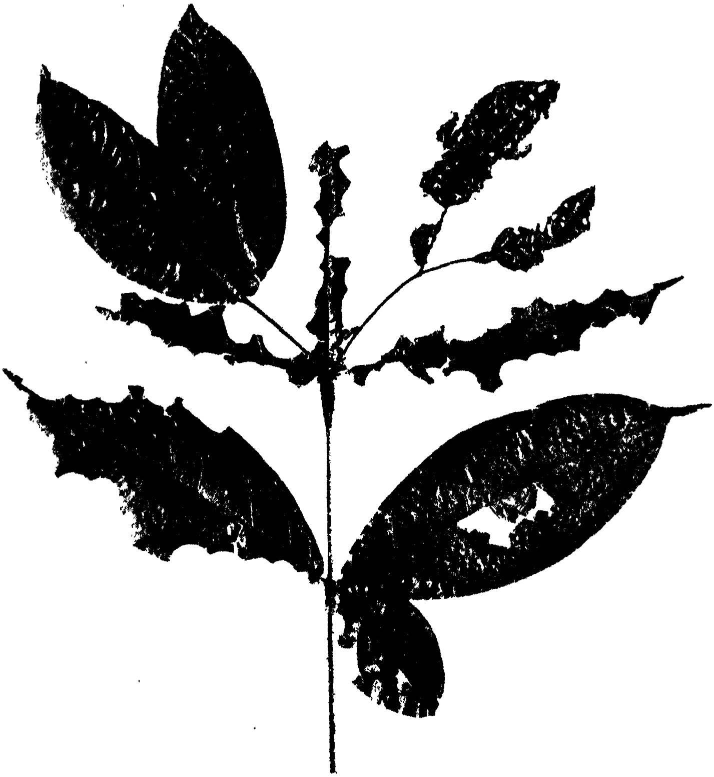

FIG. 5.—*Cordia nodosa* inhabited by *Allomerus 8-articulatus*, but partly defoliated by Attine ants.

gaps made in these dense tissues by the ants (BAILEY 3, 4). In the myrmecodomatia of *Cordia*, as in the saccate appendages of the leaves of *Tococa*, the softer and more nutritious tissues are not completely isolated from the nesting chambers, and the coccids may readily insert their setae in portions of the walls which have not been cut into by the ants.

Present status of problem of myrmecophytism

The writer's (2, 3, 4, 5) investigations of Ethiopian and neotropical ant-plants, and a critical study of data accumulated by previous students of the problem of myrmecophytism, lead to the following conclusions.

1. The neo-Lamarckian hypotheses of SPRUCE and BECCARI, and the neo-Darwinian theory of myrmecophily formulated by BELT and DELPINO and elaborated by SCHIMPER, rest upon a series of plausible deductions or teleological inferences rather than upon extensive and critical field observations and carefully planned experimental controls.

2. There are a considerable number of tropical plants, belonging to a number of distinct families and orders, which have fistulose stems, hollow petioles, saccate leaves, enlarged stipular organs, or hollow nodal or internodal hypertrophies. In most cases these putative abnormalities are inherited structures which are formed without the intervention of parasitic organisms. Certain of these plants are provided, in addition, with extra-floral nectaries or food-bodies, that is, Perldrüsen.

3. The origin and true function of these structures are at present obscure, and deserve to be studied more intensively by plant physiologists and morphologists.

4. Insects are so abundant in tropical environments, and the competition for food and for suitable nesting chambers is so keen, that most of these structural peculiarities of plants are discovered by ants. Thus, the cauline and foliar cavities, regardless of whether they have preformed entrances or not, are found to contain more or less flourishing colonies of these ubiquitous insects. In certain cases the ants are representatives of "facultative" species which nest in various habitats, whereas in other cases they are "obligatory" guests of particular species of plants. Some of them are

more inquilines, whereas others obtain a portion of their food from the host-plants, and therefore are distinctly parasitic.

5. The myrmecodomatia of most ant-plants contain more or less luxuriant growths of fungi and numerous nematodes and coccids. The relations of these organisms to the ants and to their host plants present many interesting ecological problems.

6. The relation between the ants and the coccids and their host plants is one of the most interesting phases of myrmecophytism, and one which deserves to be studied most intensively by future investigators. Certain of the plant-inhabiting ants carefully tend the coccids and solicit and feed upon their exudates. In myrmecodomatia which at maturity are jacketed internally by compact layers of thick-walled cells, the ants excavate gaps in these dense tissues, frequently inducing the formation of a nutritive callus which is fed upon by the coccids, but not, so far as the writer has been able to determine, by the ants. Only in the case of the highly specialized *Viticicola tessmanni*, which does not tend coccids, is there evidence to indicate that the ants themselves feed upon these traumatic tissues. Are all of the coccids in myrmecodomatia introduced by the ants and carefully tended by them? This seems unlikely, for certain of the ants are not known to solicit the exudates of aphids and coccids. That the Pseudomyrminae may carve up the coccids and feed them to their larvae, even if they do not utilize them as minature milch-cows, however, has been demonstrated by the writer. It seems probable that there are various degrees of specialization in the relations between the ants and the coccids, as there are in the relations between the ants and their host plants.

7. The fungi in myrmecodomatia appear to be weeds, the aerial hyphae of which are cropped or cut back by the ants; not as food, but to prevent them from obstructing the domatia and from interfering with the brood. The spores are borne into the domatia either upon the bodies of the ants or in their infrabuccal pouches, the contents of which are voided in various portions of the formicaries.

8. The nematodes live in the detritus of the ant colonies, that is, accumulations of voided pellets, liquid feces, fragments of malaxated insects, triturated plant tissues, etc. COBB finds that certain of the

nemas are highly specialized morphologically and are adapted to feeding upon the delicate hyphae of fungi which flourish in the refuse of the ants. It remains to be demonstrated whether any of the nematodes are parasitic during their larval stages in the pharyngeal glands of the imaginal ants. It may be significant in this connection, however, that nematodes are present in the infrabuccal pellets of certain Pseudomyrminae.

9. The evidence at hand supports the conclusion that the most classical ant-plant biocoenose (Azteca-Cecropia), to which SCHIMPER devoted so much attention, is an extremely interesting case of parasitism which illustrates the remarkable adaptiveness of ants in availing themselves of the potentialities of given environments. This is shown, not only in the utilization of such structural peculiarities of the plant as the Müllerian food-bodies, prostomata, and potential domatia, but more strikingly in the structural modifications produced in the tissues of the host plant which facilitate the feeding of coccids. Thus the ants are able to obtain food from their hosts in two ways, directly from the food-bodies and vicariously through the intervention of coccids.

10. The most interesting case of parasitism is that of the highly specialized *Viticicola tessmanni* upon *Vitex Staudtii*. These obligatory guest-ants induce the formation of layers of nutritive callus, rich in protein and fats, which apparently serve as their principal source of food. In so doing they avail themselves of the structural peculiarities of their hosts in a truly remarkable manner, and exercise a very delicately adjusted control over the ontogenetic development of certain plant tissues.

11. In studying the habits of phytophagous and plant-inhabiting insects, the anatomy of the host plants deserves more careful attention than it has usually received. In the case of myrmecophytic plants, the nesting and feeding habits of the ants, and their relations to coccids and fungi in many cases are largely determined by the structure and arrangement of the various vegetative tissues during different stages in the growth and differentiation of the stem or of its appendages.

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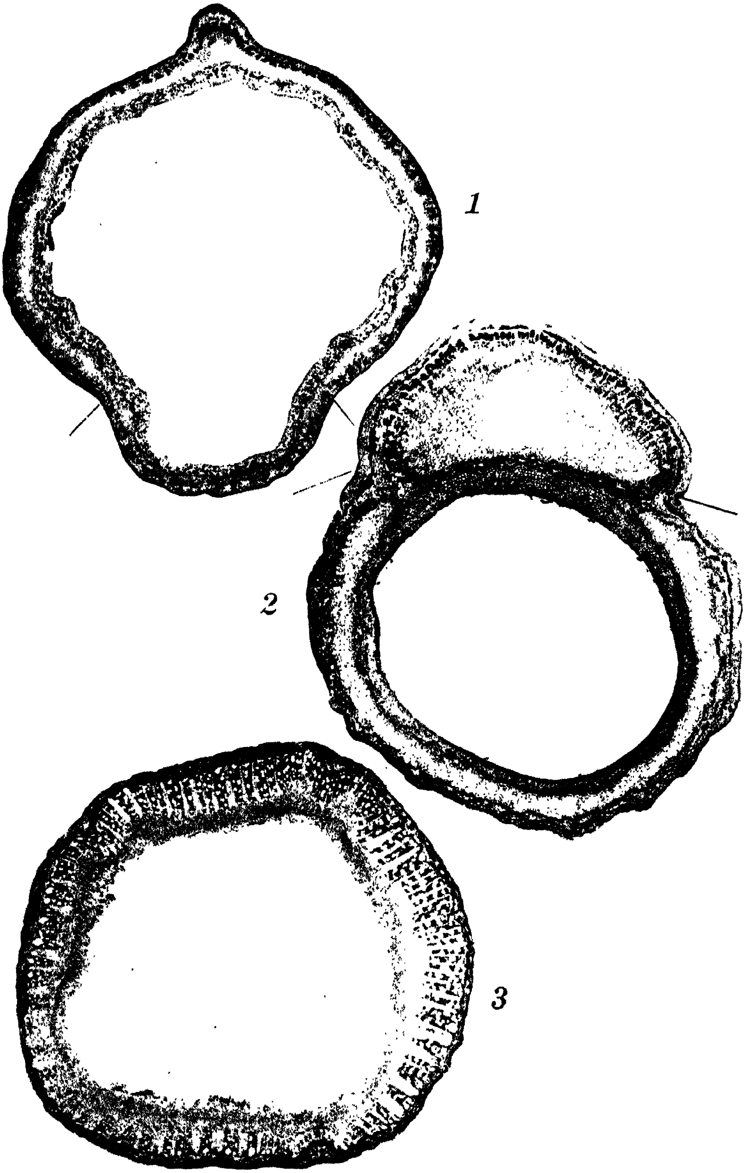

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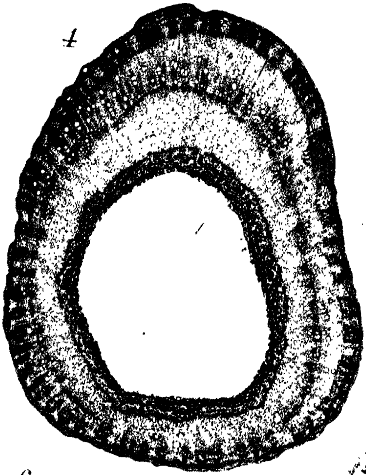

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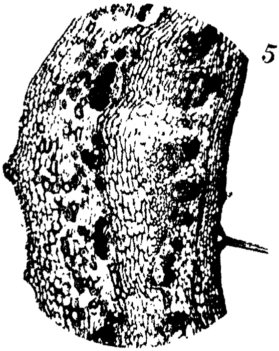

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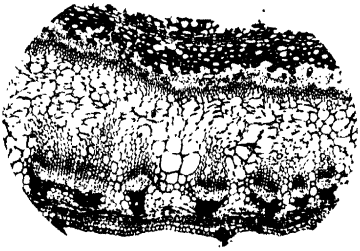

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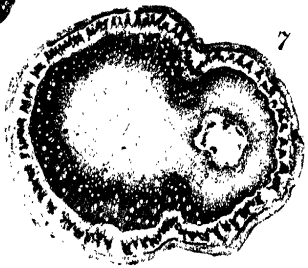

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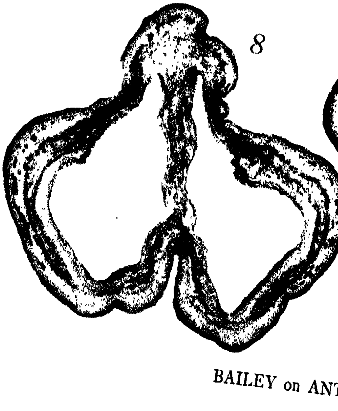

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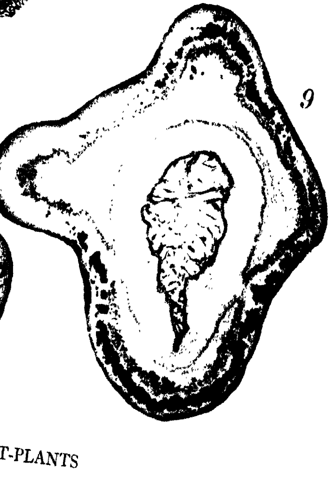

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EXPLANATION OF PLATES VI, VII

FIG. 1.—*Cordia nodosa:* transverse section of myrmecodomatium, showing centripetal and centrifugal fibrovascular cylinders; ×7.

FIG. 2.—*Cordia* species: transverse section of myrmecodomatium; inner fibrovascular cylinder is divided into two arcs which have united with two corresponding arcs of outer fibrovascular cylinder; ×14.

FIG. 3.—*Cordia Gerascanthus* Jacq. (Herbarium J. D. Smith no. 4365): transverse section of myrmecodomatium, showing medullary cavity and single fibrovascular cylinder; ×11.

FIG. 4.—*Cordia nodosa:* transverse section of base of old woody myrmecodomatium, showing inner, rudimentary fibrovascular cylinder and thick layer of xylem in outer fibrovascular cylinder; ×13.

FIG. 5.—*Cordia nodosa:* portion of fig. 1 more highly magnified; ×50.

FIG. 6.—*Cordia nodosa:* lower portion of fig. 4 more highly magnified; ×42.

FIG. 7.—*Cordia hispidissima:* (Burchell no. 9637): transverse section of base of myrmecodomatium, showing lower extremity of invagination; ×14.

FIG. 8.—*Cordia nodosa:* transverse section of immature, compound domatium, formed by invaginations from axils of two of leaves of false verticil; ×11.

FIG. 9.—*Cordia nodosa:* transverse section of apical portion of immature myrmecodomatium, showing four leaf traces and central cavity jacketed by cuticularized epidermis and numerous trichomes; ×16.

LEAVES OF TRIGLOCHIN[1]

AGNES ARBER

(WITH PLATES VIII–X)

Introduction

In a previous paper (4) a general survey was made of the leaf structure of the group of families comprised in the cohort Helobieae. While examining the leaves of one of these families, Scheuchzeriaceae (Juncaginaceae), I became interested in *Triglochin*, because this genus includes both leaves of more or less radial structure, and also typical "ribbon" leaves. The present paper describes the results of a further study of the leaves of *Triglochin*, which has been rendered possible by the generosity of J. H. MAIDEN, of the Botanic Gardens, Sydney, New South Wales, and of L. HAUMAN, of the Museo Nacional de Historia Natural, Buenos Aires, who have been most kind in supplying me with Australian and South American species. I am also indebted for material to the Director of the Royal Botanic Gardens, Kew, and to A. C. SEWARD.

Leaf structure

According to BUCHENAU (10), *Triglochin* includes two subgenera, *Eutriglochin* with united carpels, and *Cycnogeton* in which the carpels are free. *Eutriglochin*, to which the two British species *T. maritima* and *T. palustris* belong, was treated by BUCHENAU as containing twelve species, but the number has been increased somewhat in the twenty years that have since elapsed. On the other hand, *Cycnogeton* includes only one species, a native of Australasia, *Triglochin procera*. I have been able to examine this plant, and also nine species belonging to the subgenus *Eutriglochin*. In the following brief account of the leaf structure of the genus, the species, so far as possible, are taken in the order in which they are placed by BUCHENAU.

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[1] This paper represents part of the work carried out during the tenure of a Keddey Fletcher-Warr Studentship of the University of London.

SUBGENUS EUTRIGLOCHIN

TRIGLOCHIN MARITIMA L.—The leaf of this species has a sheathing base and a succulent linear limb. The sheath is continued upward into a ligule (fig. 1, *lig*). Fig. 2*A* shows the leaf sheath region in transverse section. Besides the normal bundles with their fibrous sheaths, there are occasional small strands (*f*) consisting of fibers alone. Fig. 2*B* represents a section of the limb of another leaf on a slightly smaller scale. The median bundle is placed in the usual way, with xylem above and phloem below, but the smaller peripheral bundles all have their xylem directed inward, so that in those on the adaxial side of the leaf the xylem lies below the phloem. I have indicated elsewhere (1) the reasons that lead to the interpretation of leaves showing this general type of structure as petiolar phyllodes. The relation of the bundles in sheath and limb has been described in detail by HILL (12). The changes in anatomy toward the apex of the limb of *T. maritima* can be followed in fig. 3*A*-*C*. Fig. 3*A* shows the condition reached as the tip is approached. All the lacunar tissue, which in fig. 2*B* occupies the center of the section, has disappeared, and the whole mesophyll is formed of palisade parenchyma. The vascular system is reduced until it consists of little more than the three main strands, lying in one horizontal plane. The bundle sheaths are lost, and there is a great increase of xylem relative to phloem. Fig. 3*B* shows the structure of the median bundle at a level a little lower than that of fig. 3*A*. There is a mass of primary xylem (xy_1), consisting of relatively large elements, and also a conspicuous development of secondary xylem (xy_2), composed of elements of smaller caliber, arranged very definitely in radial rows, and markedly isolated from the primary xylem. Higher up, however, there is less separation between the primary and secondary wood. The increase in the primary xylem toward the leaf apex probably is to be attributed to sliding growth. As the tip is approached, the bundles fuse into one mass (fig. 3*C*), the connection between the bundles being established by branches uniting the secondary xylem of the three bundles. Neither in the serial sections from which fig. 3*A*–*C* was drawn, nor in a longitudinal series through the apex of another old leaf, was any indication found of an "apical opening" or apical water pores. *T. maritima* thus agrees with *T. palustris*, in which BUCHENAU (10) reports the absence of an apical opening.

Fig. 4*A*, *B* shows sections of the sheath and limb of a leaf of *T. maritima* from Patagonia, for comparison with fig. 2*A*, *B*, drawn from British material. In fig. 4*A*, *B* the lacunae of the mesophyll are not outlined. The small size of the leaf (here represented on a larger scale) and the greater development both of the palisade parenchyma and fibers are very noticeable features in the Patagonian material. Instead of the occasional minute fibrous strands, which occur in the leaf sheath of the British plant, the Patagonian specimen shows six strands consisting exclusively of fibers, taking the place of the vascular bundles in the marginal region of the sheath. Without a further study of the Patagonian form, however, it is impossible to say whether these differences are constant.

In fig. 5*A*–*D* four sections are drawn, selected from a series passing upward from below through an axillary bud (*lb*) of *T. maritima*, inclosed in its prophyll (*pr*). According to BUCHENAU'S (8) observation, which REUTER (14) has recently confirmed, the lateral vegetative shoots in the axils of the upper leaves begin with a completely developed foliage leaf, while it is only the vegetative shoots in the axils of the lower leaves that have two-keeled prophylls. The bud here described belonged to the latter type. In the prophyll the main lateral bundles (*ml*) are the most conspicuous strands. Fig. 5*A* shows the origin of four of the curious "intravaginal squamules" (*a*, *b*, *c*, *d*), whose occurrence on the surface of the internode, just above the level of exsertion of each leaf, has already been recorded for this species and for *T. palustris* (13). I have discussed these squamules in a recent paper (7), and therefore now only allude to the fact that the squamule *d* is unusual in arising from the actual line of junction of prophyll and bud axis, whereas the normal state of things in *Triglochin* (the origin of the scales from the internodal surface) is exemplified in fig. 5*A* (*a*, *b*, *c*), and in α and β, belonging to the next set of squamules (fig. 5*B*). Fig. 5*B*, *C* shows that the prophyll has a closed sheathing region at the base. In fig. 5*D* the sheath is open on the side remote from the parent axis. As in the foliage leaf, there is a tendency in the prophyll for the replacement of vascular bundles by strands of a purely fibrous character. Fig. 5*E*, *F*, *G* represents three bundles from fig. 5*D* on a larger scale, to show the grades of reduction from a bundle (fig. 5*E*), which includes xylem, phloem, and a fibrous sheath, through a bundle (fig. 5*F*) in which the xylem is reduced to one element only and with little phloem, to such a strand as that shown in fig. 5*G*, which consists exclusively of a small group of fibers.

TRIGLOCHIN MARITIMA L. var. DESERTICOLA Buch.—One well marked variety of *T. maritima* is recognized by BUCHENAU. Through the kindness of Dr. HAUMAN I have been able to examine the leaf structure of this variety from Las Cortaderas, Cordillères de la Rioja. It has, he tells me, the habit of a compact "cushion plant." Fig. 6*A*, *B* shows the anatomy of the leaf sheath and limb in this variety. No definite palisade parenchyma was visible, but this may have been due to the difficulty of getting complete recovery of the tissues in dried material. The most interesting feature of the anatomy is the coexistence, in the same leaf limb, of bundles showing two types of structure, collateral and amphivasal. The more important bundles are of the normal type, but in some of the smaller strands the xylem surrounds the phloem. Fig. 6*C* shows the two bundles (marked *x* in fig. 6*B*) on a larger scale. The lefthand bundle is collateral, with a fibrous sheath (*f*) on the phloem side, while in the righthand bundle there is a discontinuous wreath of tracheids inclosing the phloem.

TRIGLOCHIN CONCINNA Davy.—This species is closely related to *T. maritima*. Its leaf structure is shown in fig. 7. The small peripheral bundles all have xylem directed inward, and succeeded externally by phloem and a patch of fibers. The xylem forms a crescent, or even an almost complete ring, including the phloem.

TRIGLOCHIN PALUSTRIS L.—This species closely resembles *T. maritima* in the main lines of its leaf structure. Fig. 8 shows the transverse section of the limb. At first glance the small peripheral bundles look almost as if they were amphivasal, but in reality there is a crescent of xylem on the inner side, and the ring is completed by fibers on the outer side. Fig. 9 represents a transverse section of the limb of a leaf of the same species from the Andes.

The leaf is smaller and the bundles are fewer than in the English material. Moreover, in the leaf from the Andes the mesophyll is all lacunar, without differentiated palisade parenchyma, and the fibers surrounding the bundles have thicker walls. A characteristic feature of *T. palustris* is the occurrence of stolons (fig. 10*A*), each of which bears scale leaves and terminates in a small bulb (**8**). This is formed of a thickened leaf (fig. 10*D*) inclosing a series of smaller leaves, also thickened and containing starch. The entire mesophyll of the leaf represented in fig. 10*D*, and even the epidermis on both sides, consists of cells densely packed with starch grains. The storage leaves are inclosed in thin scale leaves (fig. 10*B*), the veins of which are very fibrous. One of the strands toward the margin of the thin scale leaf is shown enlarged in fig. 10*C*; it is composed exclusively of fibers. The upper epidermis is strongly thickened, and the walls communicating with neighboring elements are pitted. Both the storage and outer scale leaves of the bulb correspond, morphologically, with the sheathing region of the ordinary foliage leaf, without its limb.

TRIGLOCHIN STRIATA Ruiz et Pav.—Fig. 11 shows a transverse section of the leaf limb of a specimen of *T. striata* from Natal. The small bundles toward the adaxial side have the xylem directed downward. In fig. 12 a corresponding leaf limb of *T. striata* var. *a triandra* from New Zealand is represented. In this case there are no inverted bundles toward the adaxial side of the leaf. Fig. 13*A*–*C* shows the leaf structure of another variety, *T. striata* var. *β montevidensis*. The sheath (fig. 13*A*) is succeeded by a limb (fig. 13*B*) in which, in the case of the leaf drawn, two of the minor bundles (*x*) are amphivasal. Fig. 13*C* shows one of these amphivasal bundles on a larger scale. An example of this variety, figured by SEUBERT (**16**), has ribbon leaves, and in the specimen that I examined, the limb, so far as could be told from the herbarium material available, was also ribbon-like, as is shown by the somewhat flattened form of the transverse section (fig. 13*B*).

TRIGLOCHIN BULBOSA L.—This species is widely distributed in the Mediterranean region, and also occurs in South Africa. Most of the leaves have the usual sheathing base, succeeded by a narrow limb, but some are reduced to leaf sheaths alone. Herbarium specimens have been seen with leaves at least 7 cm. long. Fig. 14*A–D* shows a series of transverse sections of a normal foliage leaf, from Crete, passing from the extreme top of the sheath (*A*) to near the apex of the limb (*D*). At the level of *D*, which is close to the tip of the leaf, the main laterals are much richer in xylem than the median bundle. The latter, however, is distinguished from the strands on either hand by the presence of a sclerized bundle sheath. Fig. 15*A* shows a transverse section of another leaf, on a larger scale than fig. 14. The three main bundles are of the normal collateral type, but in the case of the smaller bundles the xylem more or less completely surrounds the phloem. Fig. 15*B* shows the group of bundles inclosed in the dotted line in fig. 15*A*, more highly magnified. The largest bundle (one of the main laterals, *ml*) has an ordinary *V*-shaped xylem group, and is inclosed in a fibrous sheath (*f*), while each of the four small bundles (*x*) have a ring of xylem surrounding the phloem. Fig. 16*A* shows the transverse section of the limb of another leaf, in which there is a median bundle (*mb*), two main laterals (*ml*), and four minor amphivasal bundles (*x*). One of these amphivasal strands is shown on a larger scale in fig. 16*B*. To try to discover the mode of origin of the amphivasal structure, serial sections were cut through the leaf sheath of *T. bulbosa*. Fig. 17*A*, *B*, *C* shows a bundle, which, when followed in this way, at first proved to be collateral (fig. 17*A*). As the upper limit of the sheath was approached, however, the xylem elements crept round the phloem, until finally they completely inclosed it (fig. 17*C*). Another point, studied by means of serial sections of the leaf of *T. bulbosa*, is the behavior of the bundles in the apical region of the limb. Fig. 18*A* shows that, as the apex is approached, there is a tendency for the bundles to take up a position in one horizontal plane, while the individual strands become more or less amphivasal. The bundles next merge into an irregular dorsiventral complex, with the phloem chiefly on the lower side. By the level reached in fig. 18*B* (which is on a larger scale than fig. 18*A*), the phloem appears to have died out. Fig. 18*C–E* shows the gradual disappearance of the tracheids, the extreme apex of the leaf consisting of mesophyll and epidermis alone. A longitudinal section of a similar leaf tip is seen in fig. 19.

TRIGLOCHIN LAXIFLORA Guss.—This species is closely related to *T. bulbosa;* in fact, it is regarded by BUCHENAU (9) as merely a "petite espèce," which has become separated from *T. bulbosa* by difference in flowering time. Its leaf structure (fig. 20*A*, *B*) so far as can be told from examining a fragment of one leaf, shows no essential difference from *T. bulbosa*, but the palisade tissue is more conspicuous than in the latter species, and no amphivasal bundles have been observed. The leaves, like those of *T. bulbosa*, are sometimes reduced to leaf sheaths without limbs.

TRIGLOCHIN STOWARDII N.E.Br., T. ANDREWSII N.E.Br., and T. MINUTISSIMA F. v. Muell.—These three small species from Australia agree in possessing a very slender leaf limb and a delicate skeletal system. In each case I have only been able to examine one leaf. In *T. Stowardii* (fig. 21) the median bundle has a sheath in which the elements are strongly thickened on the inner side, but no such thickened sheath occurs in the case of the other bundles. In *T. minutissima* (fig. 23) there are a few fibers, not very thick walled, associated with the bundle. *T. Andrewsii* (fig. 22) has the most delicately organized leaf met with in *Eutriglochin;* the only sclerized tissue is the epidermis, whose outer wall is thickened.

SUBGENUS CYCNOGETON

Triglochin procera is an aquatic plant, occurring in Australia and Tasmania. The leaves are of the ribbon type, and may attain considerable length. Among some material kindly secured by J. H. MAIDEN from Glenfield, George River, near Sydney, New South Wales, there was more than one leaf over a meter long. Fig. 24 shows the junction of sheath and limb in one of these ribbon leaves. The ligule, which occurs in *Eutriglochin* (for example, *T. maritima*, fig. 1), is absent. The sheath wings (*sh*) are membranous, and such vascular tissue as they contain is comparatively inconspicuous. In their structure the leaves of *T. procera* show a great range of variation. Fig. 25*A*–*E* represents a series of transverse sections from below upward through a leaf of a plant from Glenfield, in which the ribbon leaves were large and strongly formed. Fig. 25*A* represents the basal sheathing region. The narrow band of mesophyll between the upper epidermis and the lacunae consists of cells not elongated at right angles to the surface of the leaf. On the other hand, in fig. 25*B*, which represents the main part of the leaf, the palisade is several layers deep, but the individual cells are not greatly elongated. There are about half a dozen main bundles, and also a large series of smaller bundles, placed nearer the adaxial surface of the leaf, just below the palisade. All the bundles are normally orientated. Fig. 25*C–E* shows sections near the apex. The zone (*m*) in *C* is a band of mesophyll tissue which has lost most of its palisadic character. At this stage there is an increase in the amount of xylem, and most of the bundles are cut obliquely in transverse sections, since they are beginning to converge. Fig. 25*D* shows considerable bundle fusion, and a further development of wood. In fig. 25*E* the bundles have united into two elongated vascular bands, consisting chiefly of tracheal elements. As in *T. maritima* and *T. bulbosa*, no sign of any apical opening was found. The stomates of the leaf epidermis are seen in fig. 26. Fig. 29*A–D* shows a somewhat different type of leaf belonging to the same species; the specimen in question came from Manly, New South Wales. The limb (*C*) is less conspicuously of the ribbon type, and the section represented included four irregular series of bundles. One bundle on the adaxial side (*ib*) was oriented with the xylem downward. Fig. 28*A*, *B* shows yet a third variation in leaf structure within the same species. These represent the sheath and limb of an extremely small and delicate ribbon leaf from Crawford River, New South Wales. The limb (*B*) has no differentiated palisade tissue, and is traversed by one series of bundles only. Details of the leaf bundles of *T. procera* are shown in figs. 27, 29*B*, *D*. As in *Eutriglochin*, the bundle sheath, in cases where it is one layered, consists of elements with a thickened inner wall (figs. 27 and 29*D*).

Comparison and Summary

AMPHIVASAL BUNDLES.—As regards vascular anatomy, the most noticeable point is the occurrence of amphivasal structure ("disposition périphérique" of CHAUVEAUD 11) in some of the minor leaf bundles of certain species of *Eutriglochin*. These amphivasal bundles, in which the tracheal elements inclose the phloem, have been observed in *Triglochin bulbosa* (figs. 15*A*, *B*, and 16*A*, *B*, 17*A–C* and 18*A*); *T. maritima* var. *deserticola* (fig. 6*B*, *C*); and *T. striata* var. *β montevidensis* (fig. 13*B*, *C*). Similar amphivasal strands have been observed in the upper part of the coleoptile in *Avena sativa* and certain other grasses, and in the scutellum of *Hordeum vulgare*. In these cases the bundles are collateral at the base, becoming concentric higher up by the creeping of the xylem elements round the phloem. The amphivasal bundles of the *Triglochin* leaf seem to have a similar mode of origin (fig. 17*A–C*). Concentric bundles are a familiar feature of monocotyledonous rhizomes, and they have been described by HILL (12) for the axis of *Triglochin*. They are also known from the scutellum of *Zea Mays* (15), but, so far as known, there has not hitherto been any record of their occurrence in the foliage leaves of monocotyledons.

FIBROUS STRANDS.—Another point in the vascular anatomy of *Triglochin* is the occasional replacement of normal bundles by strands consisting exclusively of fibers. Such strands are to be found in the scale leaves inclosing the bulbs of *T. palustris* (fig. 10*B*, *C*), and may also be observed in the base of the foliage leaf of the British *T. maritima*. In material of *T. maritima* from the Andes, however (fig. 4*A*), they are developed on a more conspicuous scale, and it is clear from their position that they are degraded representatives of the bundles which, in the case of the British plant, have a normal equipment of xylem and phloem. Fig. 5*E*, *F*, *G* shows gradations between a normal vascular bundle, in which the fibers merely form a sheath on the phloem side, to a strand consisting exclusively of fibers. The bundles in question occurred in a prophyll of *T. maritima*, but such gradations are not peculiar to this genus, and can be paralleled in other monocotyledonous leaves (1).

STRUCTURE OF LEAF APEX.—I have made some study of the anatomical changes met with in certain species of *Triglochin* as the apex of the limb is approached. These changes are illustrated for *T. maritima* in fig. 3*A–C*, for *T. bulbosa* in figs. 18*A–E* and 19, and for *T. procera* in fig. 25*C–E*. A marked feature in these three cases is the relative increase in the development of xylem as the apex is approached. Increase in wood and sclerized tissue at the extreme apex of the limb, indeed, is a common phenomenon in monocotyledonous leaves (**3**, **5**). In *Triglochin* at least, it seems impossible to interpret this excessive xylem development on teleological lines, since there is no indication of any "apical opening," or of terminal water pores. In the case of *T. maritima*, secondary xylem is formed in the apical region on a scale which is unusual for the foliar bundle of a monocotyledon (fig. 3*B*). The ultimate fusion of the bundles is brought about by tracheal branches which pass from one of the masses of secondary xylem to the next. In previous papers (**2**, **6**) it has been shown that in the leaves of monocotyledons the tracheal elements of the cross connections, uniting the longitudinal veins, tend to be attached exclusively to the secondary xylem of the longitudinal bundles, in cases in which a distinction between primary and secondary wood can be recognized. The behavior of the xylem in the leaf tips of *T. maritima* thus furnishes another example of the part played by secondary xylem in lateral connections between the foliar bundles of monocotyledons.

PROPHYLLS AND "SQUAMULAE INTRAVAGINALES."—Although previous investigators have described the prophyll of the lateral bud (**8**, **14**) and the origin of the "squamulae intravaginales," which are found among the young leaves of *Triglochin* (**12**, **13**), these structures have not been figured to show their anatomical relations, so I have drawn transverse sections of a lateral bud of *T. maritima* at different levels (fig. 5*A D*), since these sections include both the organs in question. The squamules have been discussed more fully elsewhere (**7**).

RIBBON LEAVES.—The leaves of *Triglochin striata* var. *montevidensis* (*Eutriglochin*) and of *T. procera* (*Cycnogeton*) belong to the "ribbon" type. I have not been able to make a thorough examination of the former, but have had the opportunity of cutting sections of a number of specimens of *T. procera* from different localities. The leaves of this species, although conforming to the general aquatic ribbon type, present a series of structural variations, which, however, appear to grade into one another. Fig. 25 shows a very well developed ribbon leaf, with adaxial palisade parenchyma, and numerous bundles in two series. The leaf probably floated upon the water surface. Fig. 28*A*, *B* represents a more delicate ribbon leaf, which most likely was entirely submerged. It has no palisade parenchyma, and the vascular system of the limb, at the level shown, consists of six bundles in a single series. It closely resembles a small ribbon leaf of *Sagittaria sagittifolia* (4). On the other hand, fig. 29*A*, *D* shows a solider type of leaf, whose structure recalls that of the Sagittarias of the *teres* group (4), or of the petioles of those leaves of *S. sagittifolia* which are transitional in structure between ribbon leaves and leaves with a differentiated limb (4). The parallelism between the ribbon leaves in the Scheuchzeriaceae and Alismataceae, referred to in a previous paper (4), thus finds expression in the anatomy as well as in the more obvious external characters.

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EXPLANATION OF PLATES VIII–X

Throughout, xylem (*xy*) shown in black, and in high power drawings walls of elements indicated in solid black; phloem (*ph*) in white; fibers (*f*) dotted, and in high power drawings thickness of wall indicated by double lines; *ep*, epidermis; *hyp*, hypoderm; *pal*, palisade parenchyma; *ib*, bundles with xylem directed downward; *mb*, median bundle; *ml*, main lateral bundle; *lac*, lacunae, the outlines of which are in some but not all cases indicated by dotted line. Since majority of sections were cut from dried material, there is often some distortion owing to incomplete recovery of original form, so that the transverse section is more contracted and less symmetrical than it would have been in life.

PLATE VIII

FIGS. 1–5.—*Triglochin maritima.* Fig. 1: sheath (*s*), and base of limb (*l*), with ligule, *lig* (slightly reduced). Fig. 2: *A*, transverse section of leaf sheath from Norfolk (×23), *f*, small strands consisting of fibrous elements alone; *B*, transverse section of limb of another leaf from Norfolk (×14). Fig. 3 *A–C:* transverse sections from microtome series, from below upward, through apical region of old leaf from Wells, Norfolk; *A* (×23); *B*, median bundles of section a little below *A* (×318), xy_1, primary xylem, xy_2, secondary xylem; *C*, transverse section close to apex (×23). Fig. 4 *A*, *B:* transverse section of sheath (*A*) and limb (*B*) of leaf from Patagonia (×47). Fig. 5 *A–D:* series of transverse sections from below upward through lateral bud (*lb*) with its prophyll (*pr*), and first leaf after prophyll (l_1). Parent axis would lie toward foot of page. "Squamulae intravaginales" (*a*, *b*, *c*, *d*, *α*, *β*) shaded. Fig. 5 *E–G:* three of bundles from *D* (×318); *E* is bundle marked with arrow on lower side of section; *F*, bundle marked *x*; *G*, bundle marked with arrow on upper side of section, consisting exclusively of fibers.

FIG. 6 *A–C*.—*Triglochin maritima* var. *deserticola* (co-type from Herbarium of University of Cordoba): *A*, transverse section of sheath (×47); *B*, transverse section of limb (×47); two bundles (*x*) in *C* show amphivasal character of that to the right hand (×318).

FIG. 7.—*Triglochin concinna*, California, Kew Herbarium: transverse section of limb (×47).

PLATE IX

FIGS. 8–10.—*Triglochin palustris.* Fig. 8: transverse section of limb of leaf from Surrey (×23). Fig. 9: transverse section of limb of leaf from Andes (×23). Fig. 10: *A*, base of plant (×0.5), Kew Herbarium, with two stolons (*st*) terminating in bulbs (*b*), other stolons omitted, *l*, young leaves, *fi*, fibrous remains of older leaves; *B*, transverse section of sheathing leaf inclosing bulb (×14); *C*, region including strand (*f*) in *B* (×318), *ue*, upper epidermis, *le*, lower epidermis; *D*, transverse section of thickened leaf of bulb (×14).

FIG. 11.—*Triglochin striata:* transverse section of limb of leaf from Natal, Kew Herbarium (×23).

FIG. 12.—*Triglochin striata* var. *α triandra:* transverse section of limb of leaf from New Zealand, Kew Herbarium (×23).

FIGS. 13 *A–C*.—*Triglochin striata* var. *β montevidensis*, from Mendoza, Argentina: *A*, transverse section of leaf sheath (×47); *B*, transverse section of limb of another leaf (×47), bundles (*x*) are amphivasal; *C*, amphivasal bundle from section similar to *B* (×318).

FIGS. 14–19.—*Triglochin bulbosa.* Fig. 14 *A–D*, material from Crete, Kew Herbarium, series of transverse sections from extreme top of sheath (*A*) to near apex (*D*) (×23). Fig. 15 *A*, *B*, transverse sections of another leaf from Kew Herbarium; *A*, transverse section of limb, including ten amphivasal bundles (×47); *B*, group of bundles inclosed in dotted line in *A* (×193); tracheal elements and fibers alone represented. Fig. 16: *A*, transverse section of limb of another leaf (×14), four bundles (*x*) are amphivasal; *B*, one of amphivasal bundles in *A* (×318). Fig. 17 *A*, *B*, *C*, a minor bundle followed upward from below in serial sections through leaf sheath (×193). Fig. 17 *C*, near upper limit of sheath, xylem has inclosed phloem, tracheal elements are indicated, but phloem is merely outlined. Fig. 18 *A–E*, transverse sections from microtome series passing upward from below through apex of leaf; *A* (×47); *B–E* (×77). Fig. 19, radial longitudinal section from microtome series passing through leaf apex (×318).

PLATE X

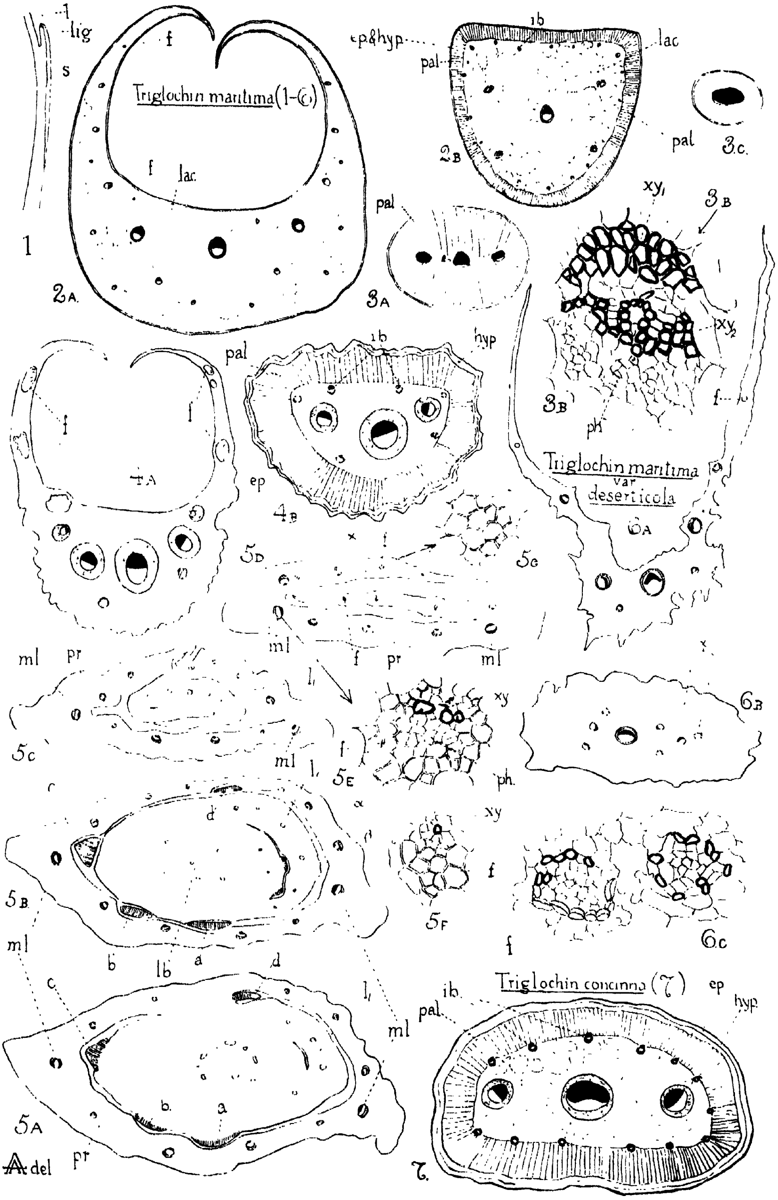
FIGS. 20 *A*, *B*.—*Triglochin laxiflora:* two transverse sections of one leaf from Malta, Kew Herbarium (×23); *B*, nearer apex.

FIG. 21.—*Triglochin Stowardii:* West Australia, Kew Herbarium; transverse section of limb of leaf (×47).

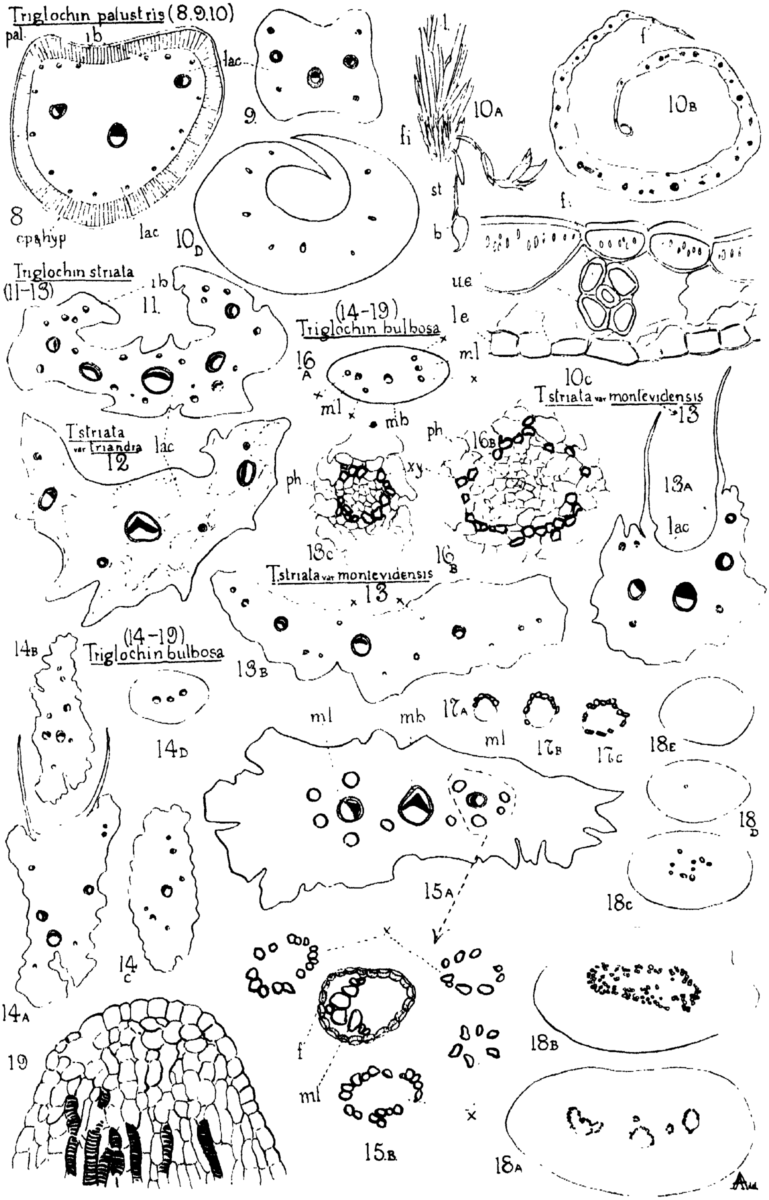
FIG. 22.—*Triglochin Andrewsii:* West Australia, Kew Herbarium; transverse section of limb of leaf (×47).

FIG. 23.—*Triglochin minutissima:* George Town, Kew Herbarium; transverse section of limb of leaf (×47).

FIGS. 24–29.—*Triglochin procera.* Fig. 24, junction of sheath and limb of leaf from Glenfield, George River, near Sydney (rather more than ×0.5); *sh*, sheath wings. Fig. 25 *A–E*, series of transverse sections from below upward, through leaf from Glenfield (×14); *A*, basal sheathing region; *B*, main part of ribbon limb, divided because too wide for page. Fig. 26, epidermis and stomates of leaf from Glenfield (×193); guard cells dotted for distinctness. Fig. 27, median bundle from transverse sections of leaf (locality unknown); *px*, protoxylem space; *bsh*, bundle sheath (×193). Fig. 28*A*, *B*, transverse sections of leaf from Crawford River, New South Wales; *A*, sheath (×14); *B*, limb (×23). Fig. 29 *A–D*, transverse sections of leaf from Manly, New South Wales; *A*, *C*, sheath and limb (×23); *B*, bundle marked (*x*) in *A* (×193); *D*, marginal bundle, such as that marked (*x*) in *C* (×193).



ARBER on TRIGLOCHIN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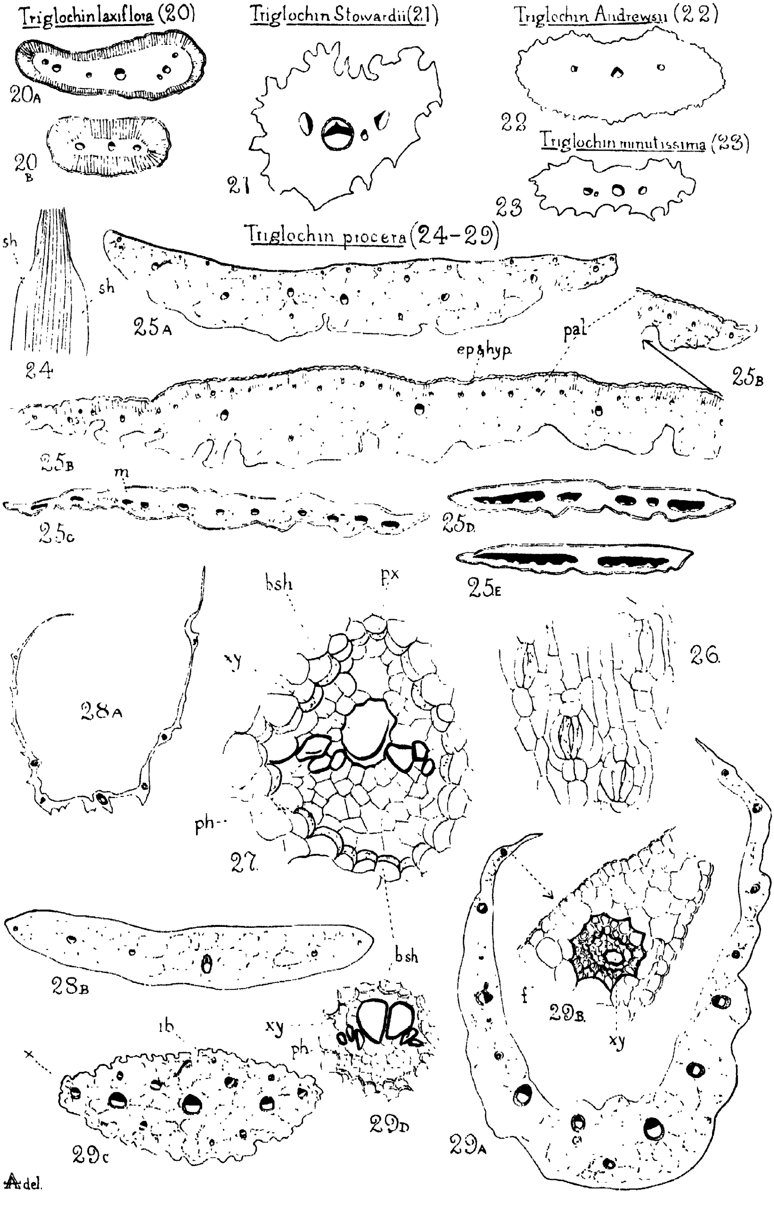
Triglochin laxiflora (20)
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Triglochin minutissima (23)
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Triglochin procera (24–29)
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ARBER on TRIGLOCHIN

INFLUENCE OF SODIUM ARSENITE ON MICROFLORA OF SOIL

J. E. GREAVES AND E. G. CARTER

Arsenic is present in many rocks and waters (14), and it has been found in varying quantities in a number of virgin prairie soils (2, 12), and soils of the arid regions often contain it. Some cultivated orchard soils contain it to the extent of 138 parts per million (5). The quantity of water-soluble arsenic in such soils varies with the total arsenic applied, and also with the quantity of alkali salts present. There is also being applied to soils in some districts arsenites (4) which are conceded to be much more toxic to plants than are the arsenates (21). The results obtained in a study of the influence of the arsenates on the microflora of the soil have been published (6, 7, 9), hence it is the province of this paper to consider the influence of sodium arsenite upon the soil microflora.

Method

The work was conducted on three different soils. Soil no. 1 was a fine sand of sedimentary origin and well supplied with all the essential plant elements except nitrogen, which was low. It contained a liberal supply of calcium and magnesium carbonate, but the organic content was very low. Soil no. 2 was a calcareous silt loam (10) which was exceptionally rich in phosphorus and potassium, but low in nitrogen and organic matter. The calcium and magnesium contents were exceptionally high, and for this reason one might conclude that the soil was unproductive. Just the reverse is true, however, for the soil was very fertile, and even with its low nitrogen and organic content produced excellent crops. Soil no. 3 was the same type of soil as no. 2, but had received fifteen tons of manure annually for the last fifteen years.

Number of organisms

The various quantities of sodium arsenite were added from a standard solution to 100 gm. portions of the different soils. These were incubated at 28°–30° C. for ten days and then plated on synthetic agar having the following composition: distilled water, 1000 cc., dextrose, 10 gm., dipotassium phosphate, 0.5 gm., magnesium sulphate, 0.2 gm., powdered agar, 20 gm. Dilutions were made of 1 to 20,000 and 1 to 200,000, incubated for seven days, and then counted. No attempt was made to differentiate bacteria from fungi, but all were listed together as total numbers of colonies.

The ammonifying powers of the soils were determined by incubating 100 gm. of the soil with 2 gm. of dried blood to which the requisite quantity of sodium arsenite had been added. The moisture was kept at optimum and at the end of four days the ammonia determined. The nitrifying powers were determined in a similar manner, except that the samples were incubated twenty-one days and the nitrates determined by a modified Ulsch method (11). The numbers of organisms found in the three different soils with and without sodium arsenite are given in table I. The results as reported are the averages of four or more determinations. The untreated sandy soil contained 1,000,000 microorganisms which were capable of developing on synthetic agar. After one part per million of arsenic was added to this soil the number was doubled. By the time 110 parts per million of arsenic had been applied, the bacterial numbers had reached 4,000,000. At the highest concentration tested the numbers had increased eight times.

The unmanured loam receiving no arsenic contained even fewer bacteria that would develop on synthetic media than did the sandy soil. The application of sodium arsenite to this soil greatly increased the total numbers, so that by the time 200 parts per million had been applied to the soil the bacterial numbers had been increased thirty times.

The manured soil untreated with arsenic contained many more organisms than did the unmanured soil. The increase in number due to the application of sodium arsenite was about the same as the increase in the sandy soil. In the sandy soil there was an increase of 800 per cent due to the arsenic, and in the manured soil an increase of nearly 700 per cent. The unmanured soil gave an increase much greater than either of the others. The nature of the curves which one obtains from the plotting of these results is quite similar in all three cases. There is an upward trend in all, which is very great in all cases where approximately 100 parts per million of arsenic had been applied to the soil. There is no downward trend in any of the curves, even when 200 parts per million of soluble sodium arsenite had been applied. This raises the question as to how much sodium arsenite may be applied to these soils before it would become toxic. It may be that the water-soluble arsenic does not rapidly increase in any of the soils, as there would undoubtedly be reactions between the calcium, iron, and other constituents, with the formation of difficultly soluble arsenic compounds and the production of sodium carbonate, which in a measure may account for the increased number. This, however, does not account for the great increase noted.

TABLE I

INFLUENCE OF SODIUM ARSENITE UPON NUMBER OF BACTERIA IN SOIL (10 DAYS)

TREATMENT ARSENIC (P.P.M.)	NUMBER OF BACTERIA DEVELOPING UPON SYNTHETIC AGAR		
	Sandy soil	College farm soil unmanured	College farm soil manured
None	1,000,000	900,000	3,900,000
1	2,200,000	4,600,000	3,400,000
10	2,000,000	5,000,000	4,000,000
20	2,100,000	6,800,000	5,600,000
30	2,200,000	7,800,000	5,800,000
40	2,200,000	7,800,000	7,200,000
50	1,800,000	7,600,000	7,000,000
60	1,600,000	7,800,000	7,200,000
70	1,500,000	9,000,000	7,400,000
80	1,800,000	9,400,000	7,800,000
90	1,900,000	13,200,000	9,800,000
100	2,700,000	13,400,000	11,000,000
110	4,000,000	13,200,000	12,600,000
120	4,200,000	13,000,000	13,200,000
130	4,400,000	13,000,000	13,000,000
140	8,200,000	17,200,000	13,200,000
150	5,000,000	16,200,000	14,400,000
160	5,600,000	17,400,000	14,600,000
170	6,200,000	17,800,000	18,200,000
180	6,600,000	21,400,000	19,200,000
190	7,100,000	26,000,000	25,400,000
200	8,000,000	27,600,000	26,000,000

The results do not appear to be explained by assuming, as do RUSSELL and HUTCHINSON (18), that the soil population is complex, and that some of its numbers act detrimentally on the bacteria, and that these are more readily killed than the bacteria. We have found no initial decrease as is the case with heat and volatile antiseptics. Moreover, one would expect that if the limiting factor be protozoa, the increase would be much more rapid in the manured than in the unmanured soil, but this is not the case. It would appear, therefore, that the arsenic is a direct protoplasmic stimulant which for a time increases bacterial growth. One of us (8) has shown that in pure cultures the metabolic activity of *Azotobacter* is increased for a time. This increase is followed by a negative phase which is similar to the negative phase following the use of a protoplasmic stimulant. Probably if the bacterial flora of these soils were followed long enough they would pass back to normal, and for a time might even show a negative phase.

Ammonification

The results obtained for the same soils in ammonification tests are given in table II. These also are the averages of four or more closely agreeing determinations. The toxicity of sodium arsenite regularly increases in the sandy soil, so that when the arsenic applied has reached 80 parts per million, the ammonia found after the lapse of four days has been reduced to one-half normal. When 200 parts per million of arsenic was applied there was only 34 per cent as much ammonia as in the untreated soil. The results for the college loam are similar, except that the toxicity of the arsenic does not increase as rapidly, and it is not until 200 parts per million of arsenic is applied that ammonification is reduced to one-half normal. On the other hand, the manured college loam shows first a stimulation and then a less rapid increase in toxicity. These are the only results which could be interpreted in the light of the protozoan theory. It would seem that the increase of the organic colloidal material in the soil decreases the toxicity of the soluble sodium arsenite, through the formation of a colloidal arsenite which may be less soluble than is the sodium arsenite. If this is the case, we should probably find that the toxicity would return as the organic manures decayed.

It is interesting to note that sodium arsenite has increased the total number of bacteria from eight to thirty times, and only in four concentrations, and that only in the heavily manured college loam did the ammonifying powers increase. It cannot be argued that the ammonifying powers have been increased, and to a still greater extent the organisms which feed upon ammonia increased, as the greatest increase in numbers is not found in those soils which exhibit the greatest decrease in ammonia production. It would appear that if the arsenite has increased the number of ammonifying organisms of these soils, it has decreased their physiological efficiency to a still greater extent. If it has not increased the total number of ammonifiers in these soils, it makes one ask what groups were increased. GREEN and KESTELL (12) found that members of the *subtilis* group were very sensitive to sodium arsenite, whereas members of the *putidum* group were very tolerant, some of them growing in one per cent arsenite broth. Arsenites, therefore, may decrease multiplication in a species with a high physiological efficiency and accelerate it in a species with a low efficiency.

TABLE II

INFLUENCE OF SODIUM ARSENITE UPON AMMONIFYING POWERS OF SOIL

TREATMENT ARSENIC (P.P.M.)	MILLIGRAMS OF AMMONIA PRODUCED IN 100 GM. OF SOIL		
	Sandy soil	College farm soil unmanured	College farm soil manured
None	136.4	101.0	91.8
1	109.8	85.0	99.2
10	109.8	86.0	102.1
20	95.5	84.0	104.7
30	80.9	88.4	96.5
40	83.3	93.5	81.6
50	83.3	85.7	74.1
60	83.3	97.6	72.4
70	66.6	88.4	69.0
80	70.0	95.7	68.3
90	57.1	93.1	71.0
100	56.7	94.1	68.3
110	52.7	82.9	69.0
120	49.3	77.8	69.3
130	50.6	68.6	74.1
140	47.9	68.3	72.7
150	47.6	66.3	69.3
160	47.9	71.0	68.4
170	47.2	63.5	68.5
180	47.2	62.2	56.7
190	46.9	60.8	65.9
200	46.9	48.9	63.9

Sodium arsenite is more toxic to the ammonifiers than is sodium arsenate. For example, one part per million of arsenic in the form of sodium arsenite stimulates ammonification in the college loam. This stimulation continues until seven parts per million of arsenic in the form of sodium arsenate has been applied, whereas sodium arsenite is toxic even when one part per million is applied. In this regard the bacteria act similarly to the higher plants, that is, they are more sensitive to arsenites than to arsenates, provided the cation in combination with the arsenic is not toxic. For instance, zinc arsenite (7) is less toxic than is copper arsenate, but sodium arsenite is more toxic than is copper arsenate. If we compare the less soluble compounds, arsenic trisulphide and lead arsenate, the difference is still greater. It is conceivable that the toxicity of the arsenites may decrease after they have been in the soil some time, due either to the oxidation of the arsenite to the arsenate or through the bacteria developing a tolerance for the same (17).

Nitrification

The results for the nitrification tests are given in table III. These also are the average of three or more closely agreeing determinations. In none of the soils tested was sodium arsenite a stimulant to the nitrifiers, unless it be assumed that the great increase in numbers is due to organisms which utilized nitrates, thus preventing their accumulation. If this were the case, we should expect the greatest decrease in nitrates where the greatest increase in number is found. This is not the case. It appears, therefore, that there is no stimulation of nitrifiers by sodium arsenite. On the other hand, sodium arsenate (7) when applied in the proportion of 85 parts arsenic per million of soil increased the accumulation of nitrates in this soil by 50 per cent. Furthermore, lead arsenate, Paris green, zinc arsenite, and arsenic trisulphide all act as direct stimulants to the nitrifiers. Sodium arsenite was more toxic in the sandy soil than in the college loam, as was also the case with the ammonifiers. It thus appears that the organic colloids act as a protection to the nitrifiers as well as to the ammonifiers. Sodium arsenite is much more toxic to the nitrifiers than are the arsenates. In this respect the ammonifiers and nitrifiers are similar to the higher plants, as KNOP (15) found that arsenites were much more toxic in water cultures to maize plants than were arsenates. This is also true when the plants are grown in sand (21).

The increase in numbers without an apparent increase in the ammonifying and nitrifying powers of the soil raises the interesting question as to what species are increased. There is the possibility that the number of ammonifiers is increased and their physiological efficiency decreased. DANBENY (3) watered barley plants with a solution of arsenious acid (1 once in 10 gallons) five times in succession, and found that the crop arrived at maturity about a fortnight earlier than the untreated part of the crop, although the amount harvested was rather less. This may be what has happened with regard to the bacteria. The arsenite has hastened maturity and has correspondingly decreased the physiological efficiency, or, the

TABLE III

INFLUENCE OF SODIUM ARSENITE UPON NITRIFYING POWERS OF SOIL

TREATMENT ARSENIC (P.P.M.)	MILLIGRAMS OF NITRIC NITROGEN FORMED IN 100 GM OF SOIL		
	Sandy soil	College farm soil unmanured	College farm soil manured
None	25.9	65.8	71.4
1	19.6	62.3	69.3
10	18.9	60.9	67.2
20	18.9	60.2	66.5
30	18.2	55.2	63.0
40	18.9	51.7	63.0
50	18.2	49.5	58.1
60	16.8	49.5	56.7
70	18.9	49.5	54.6
80	15.4	47.4	49.7
90	14.7	48.1	49.7
100	13.3	46.7	49.7
110	13.3	43.9	46.0
120	11.9	44.6	45.5
130	11.0	43.9	42.7
140	10.5	43.9	43.4
150	11.0	43.0	42.7
160	10.4	43.2	41.3
170	10.5	42.5	41.3
180	9.8	42.5	40.6
190	9.8	38.9	41.3
200	9.8	38.9	39.9

arsenite may accelerate multiplication in the less efficient groups and retard it in species having a high physiological efficiency.

KNOP considered that the stimulation of plants by arsenic was due to its replacing in a measure phosphorus. STOKLASA (**20, 21**), however, found that arsenic was unable to replace phosphoric acid, the plants dying in the flower in the absence of the latter. The senior author (**8**) has shown that arsenic cannot replace phosphorus in the nutritive media of the *Azotobacter*. It may, through a chemical interchange with the insoluble phosphorus of the soil, render phosphorus soluble, but if the *Azotobacter* is seeded into silica sand, in which the phosphorus of the Ashby media has been replaced by arsenic, there is no nitrogen fixation. Furthermore, the results point to the conclusion that the arsenic is a direct protoplasmic stimulant which shows first an increase in activity, followed by a negative phase. The stimulation of higher plants by arsenates (**19**) but not by arsenites (**1**) is what we could expect from these results. Ammonification, nitrification, and azofication are largely increased by the application of arsenates to a soil; hence the available nitrogen would be proportionally increased for the growing plant. Furthermore, the increased bacterial activity would yield increased quantities of various acids, which in turn would react with the comparatively insoluble phosphorus-carrying and potassium-carrying minerals of the soil and render them available to the plant.

Summary

1. Sodium arsenite applied to a soil greatly increases the number of organisms in that soil which will develop on synthetic agar.

2. In only three dilute concentrations were the ammonifiers stimulated by the addition of sodium arsenite to a soil. The soil in which stimulation was noted was a calcareous loam high in organic content. If the number of ammonifiers in a soil are increased by the addition of sodium arsenite to a soil, their physiological efficiency is reduced to such an extent that there is a decrease in the accumulation of ammonia over that found in an untreated soil.

3. Sodium arsenite is toxic to nitrifiers when added to a soil in only one part per million, whereas even 85 parts per million of arsenic in the form of sodium arsenate stimulates.

4. Sodium arsenite is less toxic to ammonifiers and nitrifiers in a loam soil than in a sand, and still less toxic in an organic loam than in a silt loam. The organic colloid probably forms a loose chemical combination with the arsenic, thus protecting the bacterial flora against its action.

5. Although comparatively large quantities of arsenates may accumulate in a soil without injuring its beneficial microflora, only small quantities of sodium arsenite may accumulate without producing ill effects.

AGRICULTURAL EXPERIMENT STATION
LOGAN, UTAH

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PREFERENTIAL FERTILIZATION IN OENOTHERA LAMARCKIANA

HUGO DE VRIES

In the cultures of mutant races of *Oenothera Lamarckiana*, one of the most common difficulties lies in the deviations shown by the splitting phenomena from the ordinary rules. After the self-fertilization of a splitting race, or after a cross, the expected types appear, as a rule, but in unexpected numerical relations. According to the individual cases, this may be due either to a differential growth ratio of the germs within the ripening seeds, or to preferential fertilization.

The former cause prevails among crosses of the mutants *O. gigas* and *O. semigigas*, but the second seems to be of more general occurrence. The latter may depend upon differences in individual vigor of the pollen grains as well as of the ovules. Some kinds of pollen will produce tubes with a rapid growth, whereas other tubes will have a slower development, as has been shown by the researches of NILSSON (1) and RENNER (2). Some ovules may attract the pollen tubes more effectively to their micropyle than others, and this supposition may explain the deviations in those cases in which homogeneous pollen is used.

We will first consider the cross between *O. Lamarckiana* mut. *velutina* (*O. blandina*) and *O. Lamarckiana* itself, as described by the writer (3). *O. blandina* has only one kind of gamete, belonging to the type of *O. velutina*, but modified in different points, and especially in having no lethal factor. The cross was made in 1913, the first generation yielding, as was to be expected, about one-half of the type *O. laeta*, the other half belonging to the pattern of *O. velutina*. Some of the hybrids of the first group were self-fertilized. After the rule for monohybrid splittings, we should expect the progeny to consist of 25 per cent *laeta* × *laeta*, 25 per cent *blandina* × *blandina*, and 50 per cent *laeta* × *blandina*. The first combination, or *O. amphilaeta*, must result in barren seeds, on account of the lethal factor of the *O. laeta* gametes. Thus the ratio of the culture, resulting from the cross, would become two-thirds hybrids of the *O. laeta* type and one-third *O. blandina*. Instead of this, 33 per cent *O. laeta* and 67 per cent *O. blandina* were counted.

In order to explain these unexpected figures, we may assume that the pollen and the ovules of the race of *O. blandina* are more vigorous than those carrying the *O. laeta* gametes in *O. Lamarckiana*. After fertilization the *O. blandina* tubes will come first into the ovary, and the *O. blandina* ovules will attract them vigorously. The first copulations will produce almost purely *O. blandina* germs, and only later will the remainder embrace the other possible combinations also. It is evident that such a process may be influenced strongly by external conditions, and therefore that we must expect the deviations to be very variable in amount.

In analogous crosses of *O. blandina* this type not rarely reaches about 90 per cent of the living progeny of the hybrids, and this holds good for almost all the experiments made with this race (4). Whenever the differences between the expected forms among the progeny of a cross are such as to be obvious in the seed pans or in the seedling boxes, the superfluous specimens of *O. blandina* may be pulled out before transplanting into the garden, but in other cases they may considerably reduce the number of plants of the desired type on the beds, if space is limited.

The same predominance of *O. amphivelutina* fertilizations is observed in those cases where a lethal factor is present, killing the germs within the seeds. The number of barren grains must then be ascertained. *O.* mut. *oblonga* and *O.* mut. *albida* may be chosen as examples. Their formulas are (*oblonga'*+*velutina*)×*velutina* and (*albida'*+*velutina*)×*velutina*. From this we should expect about 50 per cent of barren grains in both races, after self-fertilization. The figures obtained from the harvest of 1913 and 1921 for biennial *O. oblonga*, however, were 57–62–70–82–83, giving an average of 71 per cent barren grains. For the annual plants of the same race (1911 and 1914) it was still higher. I found 67–75–94–94–95, or together 85 per cent. *O. albida* is a race of weaker constitution, and as a rule gives a still poorer harvest. I counted 84–87–89–99, or an average of 90 per cent empty seeds. So it is in numerous other instances, the O. *amphivelutina* germs occurring almost always in a greater quantity than would be expected.

On the other hand, O. *amphilaeta* germs are usually produced in too small numbers. It may suffice to give the figures for three of the newest races, the formula of which is (*mutant′*+*laeta*)×*laeta*, which would give 50 per cent of living germs reproducing the race, and 50 per cent of *laeta*×*laeta*, resulting in barren grains. The observed figures, however, were for O. *Lamarckiana* mut. *nitens* 20–35 per cent, for O. *Lamarckiana* mut. *distans* 23–32 per cent, and for O. *Lamarckiana* mut. *elongata* 31 per cent. From this we may conclude that the O. *laeta* ovules were fertilized in these cases in too small numbers. The same condition prevails in other instances. If the pollen of these races is brought on to the stigma of other types, having two kinds of ovules, the O. *amphilaeta* will also be in the minority. For example, they were reduced to 3 per cent in O. *Lamarckiana*×*elongata*, to 15 per cent in O. *oblonga*×*elongata*, and to 11 per cent in O. *oblonga*×*nitens*.

From these considerations it is clear that, in order to be compared with the relations derived from the general rule for monohybrid splitting, the figures found for the fertilization of gametes of the type of O. *velutina* should be diminished, whereas those for the group of O. *laeta* should be increased. Constitutional strength of the races used, individual vigor, and the conditions of the cultures seem to be the chief causes which determine for each case the amount of the deviation.

Besides these, internal and hereditary factors may play some part in producing this phenomenon. Especially it may be presumed that the lethal factors might be a cause of a weaker constitution, and thereby influence the degree of the deviations from the numerical expectations. In order to try this, I have chosen a hybrid race which has only gametes of the type of O. *laeta*, but some modified and the others normal, the latter having the ordinary lethal factor, whereas the first have lost it.

In a previous article (5) I have described the hereditary behavior of O. *rubrinervis*. It is a half mutant from O. *Lamarckiana*, in which the O. *laeta* gametes have been modified in such a way as to lose their lethal factor and to obtain a factor for brittleness. In the self-fertilization of this race these gametes produce brittle individuals almost without empty seeds, which have been the origin of a new race, called *O. Lamarckiana* mut. *deserens*. If this race is crossed with the parent species we get two kinds of hybrids, the composition of which may be expressed by the formulas *O.* (*deserens'*×*laeta*) and *O.* (*deserens'*×*velutina*), both of which are viable. To the former I have given a special name, *O. lucida* (5), since it has often been used in crosses or as a control for identifying the characters of other hybrids. This hybrid, *O. lucida*, evidently has the formula *deserens'*+*laeta*×*deserens'*+*laeta*, and therefore may be made use of for a comparison of the vital gametes of *O. deserens* with the lethal ones of *O. laeta*. In the present investigation, however, we are only concerned with the number of germs produced by these two forms in different crosses.

The cross *O. deserens*×*Lamarckiana* has given 18 per cent of *O. lucida* and 82 per cent of *O. subrobusta*, the latter representing the combination *O. deserens*×*velutina*. This obviously gives a confirmation of the preferential fertilization, among the uniform ovules of *O. deserens*, by the pollen tubes of the type of *O. velutina*. The specimens of *O. lucida* obtained in this experiment had 14–15 per cent empty seeds. Among their living progeny I counted in 1917 39–55 per cent of the parental type, and 45–61 per cent *O. deserens*. If we compare these figures with the hybrid formula, which would lead us to expect 25 per cent barren *O. amphilaeta* seeds, 25 per cent pure *O. deserens*, and 50 per cent of the hybrid combination, giving two-thirds *O. lucida* and one-third *O. deserens* on the beds, we see that the *O. deserens*×*deserens* have a marked advantage over the *O. deserens*×*laeta*, which may be ascribed to the greater weakness of the *O. laeta* gametes, female as well as male.

In the first place, I have studied the ovules of the hybrid *O. lucida*, fertilizing them by uniform pollen of different constitution. In *O. albida* the pollen is *O. velutina*, with its usual lethal factor. Combined with *O. lucida* it gives hybrids of the types of *O. Lamarckiana* (=*laeta*×*velutina*) and *O. rubrinervis* (=*deserens'*×*velutina*). *O. elongata*, which is a mutant from *O. simplex*, has only *O. laeta* gametes among its active pollen grains. It must produce barren grains of *O. amphilaeta* besides the maternal type (=*deserens'*×

laeta). *O. Lamarckiana* mut. *velutina* = *O. blandina* has uniform pollen without a lethal factor. It should give partly a *O. Lamarckiana*-like hybrid (*laeta*×*blandina*), as previously described (3), and partly *O. deserens'*×*blandina* or *O. subrobusta.* Lastly, *O. deserens* itself must reproduce from *O. lucida* partly the maternal and partly the paternal type. In every case the expectation from the formula would be for equal parts of both types.

The crosses were made in 1921 and sixty offspring were cultivated for each of them, comparing them during their whole lifetime (especially at the period of flowering and ripening their capsules) with analogous hybrids of known extraction. The types on the beds were those previously given, but the numerical results differed widely from the expectation. As is seen from table I, the culture on the bed of the second cross was a uniform one, whereas the others sharply showed their two types.

TABLE I

GAMOLYSIS OF FEMALE GAMETES OF *O.* HYBR. *lucida*

CROSS	PERCENTAGE	
	laeta'×mut.	deserens'×mut.
O. lucida×albida	5 Lamarckiana	95 rubrinervis
O. lucida×elongata	14 empty seeds	86 lucida
O. lucida×blandina	33 Lamarckiana	67 subrobusta
O. lucida×deserens	42 lucida	58 deserens
Average	22	78

It is evident that in the ovaries of *O. lucida* the ovules of the *O. deserens* type are far more richly fertilized than those containing the pure *O. laeta* gametes. The lethal factor of the latter is thus seen to be connected with a smaller degree of attractiveness for the pollen tubes, and this independently of the nature of the latter. Perhaps the amount of attracting substances secreted in the micropyle is different in the two cases.

We now come to the study of the pollen. In crosses with other species *O. laeta* and *O. deserens* both produce hybrids of the type *O. laeta*, the brittleness being recessive in the first generation. Empty seeds are not to be expected, and the cultures seem to be wholly uniform during the first months of their development. When the beds are flowering, however, in July and August, two types become visible, although with only small differential characters. One of them is a normal *O. laeta*, exactly the same as those from the corresponding crosses with *O. Lamarckiana*. The other has narrower leaves, broader flower buttons, and a weaker stature. It embraces, obviously, the specimens issued from the pollen grains carrying the *O. deserens* gametes. I made the crosses in the same year as the previous ones, and cultivated sixty flowering offspring of each of them in 1921. The numerical results are given in table II. In this table the second row gives the figures for the hybrids with the normal type of the corresponding *O. laeta* as obtained from *O. Lamarckiana*, whereas the third row embraces those with narrower leaves and broader buttons.

TABLE II

GAMOLYSIS OF POLLEN OF O. HYBR. *lucida*

	CROSS	PERCENTAGE	
		sp.×laeta'	sp.×deserens'
A	O. biennis×lucida	48	52
	O. biennis Chicago×lucida	70	30
	O. Cockerelli×lucida	62	38
	O. syrticola×lucida	44	56
	Average	56	44
B	O. Hookeri×lucida	17	83
	O. blandina×lucida	5	95
	O. deserens×lucida	40	60
	Average	21	79

In group *A*, the species used for the cross were small flowered ones having small styles. In these no difference between the two types of pollen tubes is shown by the figures. In the large flowered species of group *B*, however, the difference is very obvious, since the relation is found to be about one-fourth to three-fourths instead of 1 to 1. Evidently the pollen tubes of the *O. laeta* grains are far excelled by those of the *O. deserens* pollen grains, since so many more ovules are fertilized by the latter.

The results of the tables may be summarized by saying that in the hybrid *O. lucida* (*deserens*×*Lamarckiana laeta'*), the gametes with a lethal factor are less effective in fertilization than those without that character. The lethal ovules, as well as the lethal pollen tubes, contribute a smaller part to the harvest than would be expected from the ordinary rule for monohybrid splittings. In other words, the *O. laeta* gametes are weak in fertilization, not only as compared with *O. velutina*, but also in comparison with those of their own type, which lack their lethal factor. From these facts it seems probable that the cause of this weakness is connected in some way with the presence of the lethal.

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LYCOPODIUM PROTHALLIA IN WESTERN MASSACHUSETTS

ALMA G. STOKEY AND ANNA M. STARR

In 1917 SPESSARD (4) published the first account of the finding of *Lycopodium* prothallia in America. At the same time CHAMBERLAIN (3), in connection with his account of the prothallia and sporelings of three New Zealand species of *Lycopodium*, gave a historical résumé of the literature on *Lycopodium* prothallia, including accounts of collections both in the open and of cultures. SPESSARD referred his material to five species: *L. complanatum*, *L. annotinum*, *L. clavatum*, and two which had not previously been reported, *L. lucidulum* and *L. obscurum*. Later he published a note (5) stating that the specimens which he had called *L. obscurum* were *L. complanatum*, but that in his later collections he had found undoubted *L. obscurum* prothallia. In 1922 he gave a more extended account of the prothallia of *L. lucidulum* and *L. obscurum* (6).

Since October 1920, eleven stations have been found in western Massachusetts, of which seven, established by the writers and W. P. STOKEY, will be described in this article, and four will be described by DEGENER in this issue. The seven stations are as follows:

1. *L. complanatum:* Moody Corners, South Hadley.
2. *L. obscurum* and *L. complanatum:* Island in Forge Pond, Granby.
3. *L. obscurum:* Mt. Toby, Sunderland.
4. *L. obscurum*, *L. complanatum*, and *L. clavatum:* Smith Ferry Woods, South Hadley.
5. *L. obscurum:* Upper Lake Woods, South Hadley.
6. *L. obscurum:* Aldrich Mills, Granby.
7. *L. obscurum:* Dark Woods, Granby.

These stations are from one to sixteen miles apart, and are in three quite distinct types of woods. The first station was found in October 1920, and the others during the summer and autumn of 1922. A description of each station will be given, with a discussion of the conditions which were found to prevail.

Station 1, Moody Corners, South Hadley.—A colony of more than 100 sporelings of *L. complanatum* was found on the northeast facing slope of a glacial dump above a small brook, in an area of about 1.5×8 m. They were 1-2.5 m. above the level of the water. The knoll is covered with a young growth of mixed hardwoods, with a few pines, and although the growth is rather open the ground is always shaded. The trees on the slope are *Tilia americana*, *Carpinus caroliniana*, and *Fraxinus americana*, with a few seedlings of *Betula populifolia*. The ground was but partly covered with a sparse growth of *Maianthemum canadense*, *Polygala paucifolia*, a species of grass, and small patches of mosses, *Polytrichum commune* and *Catherinea angustata*. The soil was firm and compact, consisting of leaf mold, clay, and sand to a depth of 8- 10 cm., with a subsoil consisting of clay, sand, and gravel. The prothaliia were found at a depth of 0.5-3.5 cm. The smaller sporelings were attached to prothallia, and some prothallia without sporelings were found also. The prothallia were small and less symmetrical than the typical form. The sporelings were slender and fragile in appearance, but apparently healthy. The nearest spore-bearing plants of *L. complanatum* were found 25 m. to the east, but these plants appeared to be too young to have been the parent plants.

Station 2, Island in Forge Pond, Granby.—This small island, which at its highest point is not more than 3 m. above the level of the pond, has a mixed growth of hardwoods, with a few old pines at the south end and a few young pines at the north end. Several thrifty patches of sporelings of *L. obscurum* were found at the north end, on the north and northwest facing slopes, where there is a growth of *Betula populifolia*, *Acer rubrum*, and *Viburnum dentatum*. White pines were growing near this zone, but no sporelings were found where there was any noticeable deposit of pine needles. The region is well shaded but rather open, so that few leaves remain on the ground. The herbaceous growth is rather sparse and consists of small patches of *Polytrichum commune*, a few plants of *Maianthemum canadense* and *Polygonatum biflorum*, and scattered tufts of a species of grass. The top soil consists of a layer of coarse sandy loam, 6-10 cm. deep. There is a sharp transition to the underlying stratum of sand. Numerous prothallia, both with and without sporelings, were found 2–5 cm. below the surface. Only four prothallia of *L. complanatum* were found, all without sporelings, but these were larger and more symmetrical than those produced much more abundantly at Moody Corners. Only three fruiting spikes of *L. obscurum* were found on the island, but there were small patches of vigorous growth at distances varying from 2 to 6 m. from the sporelings. On the mainland, 200–250 m. from the island, there is a luxuriant growth of both *L. obscurum* and *L. complanatum*, which fruit abundantly, and a scattering growth of *L. clavatum* which was not found in fruit. Several plants of *L. clavatum* were found on the island near the patches of sporelings, but none was found in fruit and there were no sporelings of this species.

Station 3, Mt. Toby, Sunderland.—This station is on the west side of one of the smaller hills of the Mt. Toby group. The general aspect is distinctly different from that of the stations just described. There is almost no slope, no body of water, and the region has the appearance of being rather dry. The tree growth is of mixed hardwoods with a few young pines; there is little undergrowth. The soil is a rich sandy loam to a depth of 8–10 cm., with a sandy subsoil. There was a considerable deposit of dead leaves. Sporelings of *L. obscurum* were abundant, occurring in small groups, apparently limited to small shallow depressions. The prothallia were 2–6 cm. below the surface, averaging a somewhat greater depth than in the other stations. Owing to the presence of the layers of leaves, the stems of the sporelings, in addition to being unusually long, were unusually crooked. Some of the prothallia were exceptionally large, and attained a greater size than has been recorded for any subterranean species, measuring as much as 18×10 mm. Mature plants of both *L. obscurum* and *L. complanatum* were abundant in the woods but were at a considerable distance.

Station 4, Smith Ferry Woods, South Hadley.—Sporelings and prothallia of three species were found in this woods, which in general has the aspect of the Mt. Toby station. There is a young growth of *Quercus rubra* and *Q. alba*, an older growth of *Betula populifolia*, and the remains of old trees of *Castanea dentata*. There are a few young white pines. The sparse herbaceous growth in the vicinity of the sporelings consisted of *Mitchella repens* and *Polytrichum commune*. The soil is a sandy loam to the depth of 6–10 cm., with sand beneath. Comparatively few leaves collect in the depressions, to which the prothallia seem to be limited. The first sporelings found were those of *L. obscurum*, three patches with three or four in a patch. These were in a broad depression near a ridge 1–2 m. high. The prothallia to which they were attached were growing 1–3 cm. below the surface. The prothallia and sporelings of *L. complanatum* were also found in depressions, shallower, however, and about 100 m. from those of *L. obscurum*. Fifteen sporelings were found, three of which were attached to prothallia. These prothallia were symmetrical in outline and relatively large, one measuring 11×2.5 mm. They were 2.5–4 cm. below the surface. In the sporelings found in this region the characteristic habit of *L. complanatum* was later in appearing than in those at Moody Corners, the flattening of the branch and the broadening of the leaf base not being evident until after the fourth or fifth branching, while in those at Moody Corners it usually began after the second or third branching. Two sporelings of *L. clavatum* with prothallia and two without were found about 10 m. from the patch of *L. complanatum*. These two prothallia were nearer the surface than any prothallia collected, and were barely covered by the loose leaf mold at the surface. Old plants of *L. complanatum* were fruiting abundantly 25–50 m. west of the patch of prothallia. Fruiting plants of *L. obscurum* were in the same general region, but were somewhat less abundant. *L. clavatum* does not fruit freely in this part of Massachusetts, and while many vigorous old plants were found, none were seen which were bearing fruiting spikes.

Station 5, Upper Lake Woods, South Hadley.—A patch of more than 50 sporelings of *L. obscurum* was found on the more or less level region 2–4 m. from the northeast side of the lake. The bank at this place is about 2.5 m. high. Many sporelings were attached to prothallia, and several prothallia were found which had not produced sporelings. The prothallia which were distributed over an area of 2×4 m. were found 1–3 cm. below the surface. The soil is a firm sandy loam to a depth of 7–10 cm., over a sand and gravel subsoil. The region is well shaded, chiefly by a large

Acer saccharum, but it is rather open and few leaves remain on the ground. The other woody growth is *Betula populifolia*, *Hamamelis virginiana*, and young *Quercus rubra*. The soil is partly covered with a species of grass and occasional small patches of *Leucobryum glaucum* and *Dicranella heteromalla*. A few spore-bearing plants of *L. obscurum* were found about 100 m. to the northwest, and another patch was found across the lake about 200 m. to the south.

Station 6, Aldrich Mills, Granby.—The place was investigated because it is one of the few places in this region where *L. clavatum* can be found fruiting freely. No sporelings of *L. clavatum* were found, but two sporelings of *L. obscurum* with gametophytes, and two colonies of sporelings which had lost their gametophytes were found. This station is distinctly different in aspect from those previously described, but is similar to two described by DEGENER. The sporelings were growing in a dense grove of young hemlocks on the north-facing slope 0.5–2.5 m. above a small floodplain on Bachelor Brook. The soil, which was completely bare of other plants, was covered with hemlock needles, and consisted of a loose sandy loam with considerable organic matter. There were no old plants of *L. obscurum* within 40 m.

Station 7, Dark Woods, Granby.—A few sporelings of *L. obscurum* were found in a well defined depression in a rather open grove of *Acer rubrum* and *Betula populifolia* on the edge of the Dark Woods. The herbaceous growth consisted of *Pyrola rotundifolia*, *Cypripedium acaule*, small patches of *Polytrichum commune*, and a species of grass. The depression was 0.4–1.0 m. below the surrounding level. About 15 m. away, on the opposite side of a slight ridge 0.6–1.0 m. high, there is a small but permanent pond. There were a few dead leaves on the ground, covering a thin layer of leaf mold; below this was sandy loam passing gradually into pure sand. There was less organic matter in the loam than was found in the other stations. There were only two sporelings with prothallia, and these were small; there were three sporelings which had lost the prothallium but which still had the foot. The prothallia were 0.7–1.5 cm. below the surface. Old plants of both *L. obscurum* and *L. complanatum* were fruiting about 3 m. away, and other patches were abundant throughout the adjacent parts of the woods.

Discussion

The stations fall roughly into three types: (1) a grove of mixed hardwoods on a slope or near a slope above a body of water; (2) depressions in a grove of mixed hardwoods where the general aspect is that of a relatively dry woods; (3) a grove of hemlocks.

Certain conditions were found to be similar in all three types of habitat. The top soil consists of sandy loam, usually with a large amount of humus, and it is fairly compact except under hemlocks. The presence of considerable humus is doubtless important, both in relation to the moisture content of the soil and to the development of the fungus with which the prothallium maintains a symbiotic relationship. The three species of prothallia are of the subterranean type, which contains an endophytic fungus. Fungal hyphae were usually found in great abundance in the soil in which prothallia were growing. In only one station was there any considerable deposit of dead leaves. It is possible that many layers of leaves would tend to prevent the spores from washing down into the soil to suitable depths. Where the spores are deposited in large quantity this might not make any difference, but where the spore deposit is light, as at a distance of 50-100 m. from spore-bearing plants, and in cases where the percentage of germination is only 5 per cent, as BRUCHMANN (2) reported for *L. clavatum* and *L. annotinum*, the presence of many layers of leaves might be an important factor in the distribution of prothallia.

In all the regions the variation in the level of the water table is moderate. This may be due to proximity of bodies of water, or to a favorable topography in relatively dry woods such as is afforded by depressions where evaporation is less than in the surrounding areas, and where drainage from the surrounding levels tends to maintain the water supply. In the hemlock groves the dense shade and the protection given from the wind, together with the high humus content of the soil, would make the maintenance of an adequate moisture content a fairly simple problem. One explanation of the comparatively rare occurrence of prothallia and sporelings may be that, owing to their very slow growth, there is always a high degree of probability that an exceptionally dry summer will lower the water table to such an extent that prothallia and sporelings in all stages of development will be killed. It seems not improbable that crops of sporelings would be more likely to appear after a period of 10 to 12 years without a protracted drought.

All the stations were in well shaded regions. The slopes were mostly north-facing. SPESSARD found in Michigan that prothallia and sporelings grew in the open. In western Massachusetts, however, no sporelings were found in the open, and it is not usual to find old plants in sunny places. In this region there appears to be some agreement between the conditions which are favorable for the early stages of the white pine and for *Lycopodium* prothallia. The pines which were found in almost every station agree roughly in age with the *Lycopodium* sporelings, if we may assume that the prothallia and sporelings in this region develop at a rate similar to that which BRUCHMANN determined. The presence of white pines among second growth timber was found to be a good indicator. No sporelings were found in a pure stand of pine.

In all the regions studied the soil is well drained, the subsoil consisting of sand or sand and gravel. This seems to be of considerable importance, as it was found that when sporelings and prothallia were brought into the laboratory and greenhouse, the sporelings and old plants would live for many months in undrained dishes, but the prothallia would last only a few weeks under such conditions. Prothallia, however, will live in the laboratory indefinitely if kept in well drained sandy soil. They grew particularly well on the surface of sandy loam under a bell-jar. Under such conditions they develop chlorophyll. While old plants are frequently found in swampy places, the indications are that they do not start there.

The prothallia and sporelings are restricted to places where there is little or no herbaceous growth. They are often found near small patches of *Polytrichum*, but they do not seem to be able to compete with old established patches. They usually grow at some distance from old plants of *Lycopodium*, as has been noted by BRUCHMANN (1) and SPESSARD (6), although SPESSARD reports finding a few sporelings which were growing among old plants. In only two cases were prothallia and sporelings found within 3 m. of old plants; in most cases they were 15–50 m. away, and in some cases 100–200 m. away. Nothing was found which suggested the growth of sporelings in groups of four, as reported by SPESSARD, nor anything to indicate any other medium of spore dispersal than wind. In the case of station 5, which is within 5 m. of a much used path, it is possible that fruiting spikes may have been carried there by persons, but in the cases of stations 1 and 2, where the sporelings are even more abundant, it seems highly improbable. It is easier, naturally, to find sporelings when they are in groups, but so many isolated sporelings and groups of two or three were found that no basis is seen for any generalization about grouping.

Until we know something about the character of the endophytic fungus in relation to its occurrence in the soil, we are much restricted in our explanations of the conditions necessary for the development of *Lycopodium* prothallia. We do not know how many species there are, and whether they are of rare or frequent occurrence. If, as SPESSARD suggests, the various prothallia are associated with different species of fungi, we may have an explanation of the fact that in only one station, no. 2, were the prothallia of two species found closely associated. In stations 3, 5, and 7 the conditions apparently were equally favorable for the presence in the soil of spores of *L. complanatum*, and in station 6 much more favorable for *L. clavatum* than for *L. obscurum*. It may be that it is the absence of the fungus which makes the development of prothallia and sporelings a rare occurrence, but it seems equally probable that the rarity is due to the numerous contingencies of an exceptionally long infancy.

It is hoped that this analysis of the stations found in western Massachusetts may give some indications of the conditions necessary for the development of prothallia and sporelings, and also some suggestions as to situations in which one may look for them with reasonable prospect of success. It should not be assumed, however, that the requirements for germination and development will always be found under the same topographical conditions. In higher latitudes and in regions with fewer sunny days in summer, it is to be expected that sporelings will be found in places with less shade than those here described. While the moisture relations are undoubtedly complex, SPESSARD's concise directions to look for prothallia in conditions less extreme than those in which the parent plants are growing, is in line with the observations in this region.

In view of this successful search for prothallia and sporelings during the past year, it seems probable that they are not so rare as has been supposed. It is somewhat difficult to recognize the sporeling in the early stage, and they are apt to be found in what ordinarily would be considered poor collecting grounds. The chief difficulty seems to be in finding the first one. The first patch was found more or less by accident, although we had been looking for sporelings casually for the past ten years. After seeing the sporelings growing and noting the conditions which seemed to favor their development, it was comparatively easy to find other stations. It seems probable that in any place where *Lycopodium* fruits abundantly there is a fair chance of finding prothallia and sporelings if attention is given to selecting suitable places for investigation.

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FOUR NEW STATIONS OF LYCOPODIUM PROTHALLIA[1]

OTTO DEGENER

(WITH PLATES XI–XIII AND TWO FIGURES)

On March 29, 1922, while on a geological trip on East Rock Mountain, Great Barrington, Massachusetts, the writer noticed a few small lycopods growing in the shelter of a bowlder. Since these plants did not appear to be like any of the common lycopods in the region, they were removed and found to be sporelings, several of which still had the gametophytes attached. Unfortunately time did not permit a thorough investigation. The slope of East Rock where the specimens were found had been stripped of timber several years previously, so that bramble thickets had had time to form among the stumps and old laurel bushes. The soil from which the plants were taken was a medium sandy loam, thinly covered by a moss. There was little moisture in the soil, although the slightly overhanging rock no doubt hindered the drying influence of the sun except during the morning. The area that was turned over to get the plants might easily be covered by the hand. There were seven specimens, four of the sporophytes being still firmly connected with the gametophytes, and three showing only the disintegrating foot with which they had absorbed nourishment from the sexual plant.

The gametophyte strikingly resembles a carrot in shape. It is roughly conical, not counting the small crown at the top where the sexual organs are located. It is dirty gray in color and exhibits a pubescence due to rhizoids. The smallest specimen unearthed had a prothallium 2.5×7 mm., while the sporophyte had just barely reached the surface of the soil 15 mm. above. The root, arising from the sporeling above its foot, had divided three times dichotomously, even though it was no longer than the prothallium itself. Another gametophyte, more nearly the size of the other two, measured 4×10 mm. Its sporeling, which in this case had just produced two equal branches, was 30 mm. in length. There appeared to be a main root from which secondary roots had developed, through a modified dichotomy. This gametophyte was buried approximately to the same depth as the one just described.

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[1] In December 1922, after this paper had been written, the writer found hundreds of prothallia and sporelings of *L. cernuum* near the active crater of Kilauea, Hawaii. Since these plants grew under very unusual conditions, it is hoped to deal with them in a separate paper.

The remaining sporelings lay at a depth of about 3 cm. One of these was creeping just below the surface. Its prostrate stem showed no sign of changing its horizontal course, in spite of the fact that three upright shoots, each about 2 cm. in length, had formed. Another sporeling, the gametophyte of which had decayed, showed no marked creeping tendency, but had produced five branches within a distance of 2 cm. above the foot. Yet another, having two small shoots near the foot, had developed a main stem 5 cm. long. Higher up it was approaching the mature sporophyte in appearance, making the species determination certain.

The leaves of this sporeling, for instance, are scalelike at the subterranean base. A little farther up the terete stem they become awl-shaped, sometimes over 4 mm. in length, and arranged in whorls of threes that are spaced at intervals of about 2 mm. After the stem has branched six or seven times, the leaves, which are now four-ranked, assume two shapes. The lateral are more flattened, while those at right angles to them resemble more closely small juvenile leaves. The stem now appears laterally compressed because of the two ranks of wide leaves which possess decurrent adnate bases. This fact, in addition to the shape of the gametophyte attached to the other sporelings, proves the specimens to be *L. complanatum*. Because of a question raised by SPESSARD[2] in regard to the position in which the prothallia are found, it is well to emphasize the fact that the Great Barrington specimens grew with the longer axis inclined between the horizontal and the vertical positions.

On May 7, R. F. MARTIN, searching for *Lycopodium* prothallia at Orient Springs, near Amherst, Massachusetts, was rewarded by the discovery of three sporelings, one of which had an excellent gametophyte at the base. These plants were growing in a hemlock grove in moderately dry soil on the side of a ravine. Since many mature sporophytes of *L. clavatum* were growing near that spot, he inferred that his specimens belonged to the same species. Professor STOKEY identified them, however, as *L. obscurum*, the gametophyte of which had been discovered in 1917 by SPESSARD.

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[2] SPESSARD, E. A., Prothallia of *Lycopodium* in America. BOT. GAZ. 63:71. 1917.

In an entirely different locality at Amherst on June 13, the writer noticed several more sporelings of a lycopod, with gametophytes. These were identified as *L. obscurum* var. *dendroideum*, identical with those found by MARTIN. This last station is situated on a steep slope covered with large hemlocks that produce enough shade to keep the ground quite bare of plants. The soil is a moderately rich loam, underlaid at 6–8 cm. by a well drained yellow soil of similar texture. The upper layers seemed rather compact and did not crumble when dug, owing to the presence of fungal threads. Another fact possibly worthy of mention is the presence of many hemlock rootlets, the tips of which were greatly swollen with a mycorrhiza. A fungus which is probably a species of *Tremellodendron* grows well at that station. In fact, a large sporeling was found entirely surrounded by this fungus. Whether any of these have an endophytic connection with the lycopod is not known (fig. 1).

FIG. 1.—Sporelings of *L. obscurum* in hemlock grove

During the few afternoons that were available, between two and three hundred specimens were collected, varying from prothallia of about 1.5 mm. in diameter to mature ones with sporelings 10 cm. or more in length. Almost all of them were collected in a circular space less than three feet in diameter. More than two-thirds of the prothallia, according to measurements based on the sporelings, had developed at a depth of 2.5–7 cm. Many were growing at greater or lesser depths; only one, however, was noticed at 9 cm. and but a few at 1.5 cm. Because of the difficulty of distinguishing prothallia from soil particles, these figures as to the extremes of position are not as reliable as might be desired.

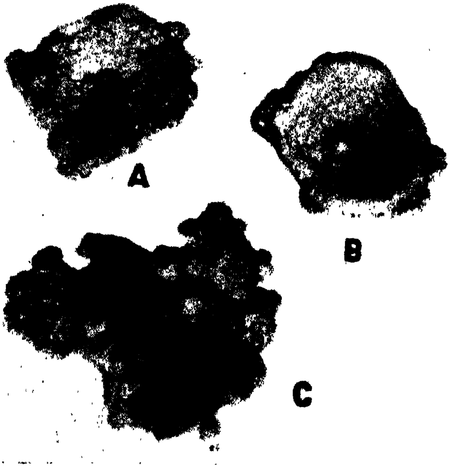

FIG. 2.—Prothallium of *L. obscurum* showing slightly depressed upper surface (*A*), prothallium showing under surface with protuberance (*B*), prothallium showing upper surface with revolute margin (*C*); ×4.5.

These plants have the saddle-shaped prothallium characteristic of such species as *L. annotinum* and *L. clavatum*. Their general contour is circular, but this shape is often modified by a later growth of unequal magnitude. The center is slightly depressed (fig. 2*A*), while in older specimens it may even be overlapped by the revolute margin upon which the sex organs are situated (fig. 2*C*). These develop intramarginally, the outer boundary of the prothallium being well defined from the smoother sterile underside. In most of the specimens a protuberance is discernible in the center of the lower surface; it is no doubt the earliest part of the gametophyte formed (fig. 2*B*). As in the case of the specimens first described, rhizoids are visible to the unaided eye. The color, however, is of a more yellowish tinge. Fuller studies on this species, with special reference to the stelar structure of the sporeling, are now in progress.

In taking the greatest diameter of 140 prothallia from which no sporelings had developed (pl. XI), it was found that about sixty measured 4–5 mm. in the greatest diameter, half that number 6–7 mm., while seven were 8 mm. and three were 9 mm. Only two were 1.5 mm. and eight were 2 mm. in diameter. The smallest gametophyte producing a sporeling was 4 mm. in diameter and the largest 10 mm. The most frequent size, however, ranged from 5 to 8 mm.

It may be well to describe how the material was procured, for this may explain why the smaller gametophytes seemed so rare in that locality. After carefully clearing away the covering of hemlock needles and twigs from the soil, a sporeling with the soil surrounding it was removed. Then the earth was broken away from the stem of the sporophyte until the prothallium was laid bare. During this process, several other prothallia might come to view. After most of the sporelings had been gathered by this method, a handful of soil was taken from the general region and placed in a sieve to be washed. After all the earth had been removed, sometimes as many as six gametophytes were to be found among the rootlets and other particles of fibrous matter remaining. That so few small specimens were found is due no doubt to the large mesh of the sieve employed. The number of gametophytes of all sizes still growing in that locality must mount up into the thousands.

The sporelings of *L. obscurum* are upright in habit, and often produce one or two subterranean shoots which appear arrested in growth (pl. XII). The main stem commonly branches two or three times before reaching a height of 5 cm. As in the preceding species, the earliest leaves below the surface of the soil are scalelike, while the later ones are linear awl-shaped, and generally spaced farther apart on the stem than the mature type of leaf.

In both of these species the gametophyte is not restricted to growth in a definite position. As already noted, the conical gametophytes of *L. complanatum* found in Great Barrington grew neither strictly upright nor strictly horizontal in the soil. Of the specimens of the same species found by SPESSARD, one was definitely known to have grown with the longer axis in a vertical position, while others grew with the longer axis horizontally directed. In mentioning a later find,[3] he definitely states that they do not all grow in the same position. Of the few saddle-shaped gametophytes of *L. obscurum* seen by the writer *in situ* at Amherst, several were growing in an inverted position. Of the sporelings that were gathered, four had developed from inverted prothallia 6–7 mm. in diameter (pl. XIII *A*, *C*), while one had grown from a gametophyte 4 mm. in diameter which was standing on its edge so that the flattened surfaces stood vertically (pl. XIII*B*). It is probable that the surface with the reproductive organs is normally directed upward, but that when displaced by any chance, a sporeling can still develop, provided fertilization occurs.

A dozen sporelings of *L. clavatum* were later discovered growing under an isolated hemlock, possibly 400 yards from the preceding station. But one specimen, 2 cm. in length, had a saddle-shaped gametophyte 4.5 mm. in diameter attached to it. This was buried at a depth of 5 mm. The others possessed a foot about 1 mm. in diameter through which the sporeling had been attached to the prothallium. In measuring the sporelings, it was evident that the prothallium in no case grew at a depth below 2 cm.

This location is very similar to the one in which the *L. obscurum* material was discovered. The ground was not sloping, however, and was sparsely covered with moss and grass. The sporelings also were growing in close proximity to one another. In the field they were easily distinguished from the sporelings of *L. obscurum* by their prostrate habit and more fuzzy appearance, since the leaf is tipped by the bristle so characteristic of that species. The subterranean and later juvenile leaves from a macroscopical examination did not differ essentially from the corresponding types described.

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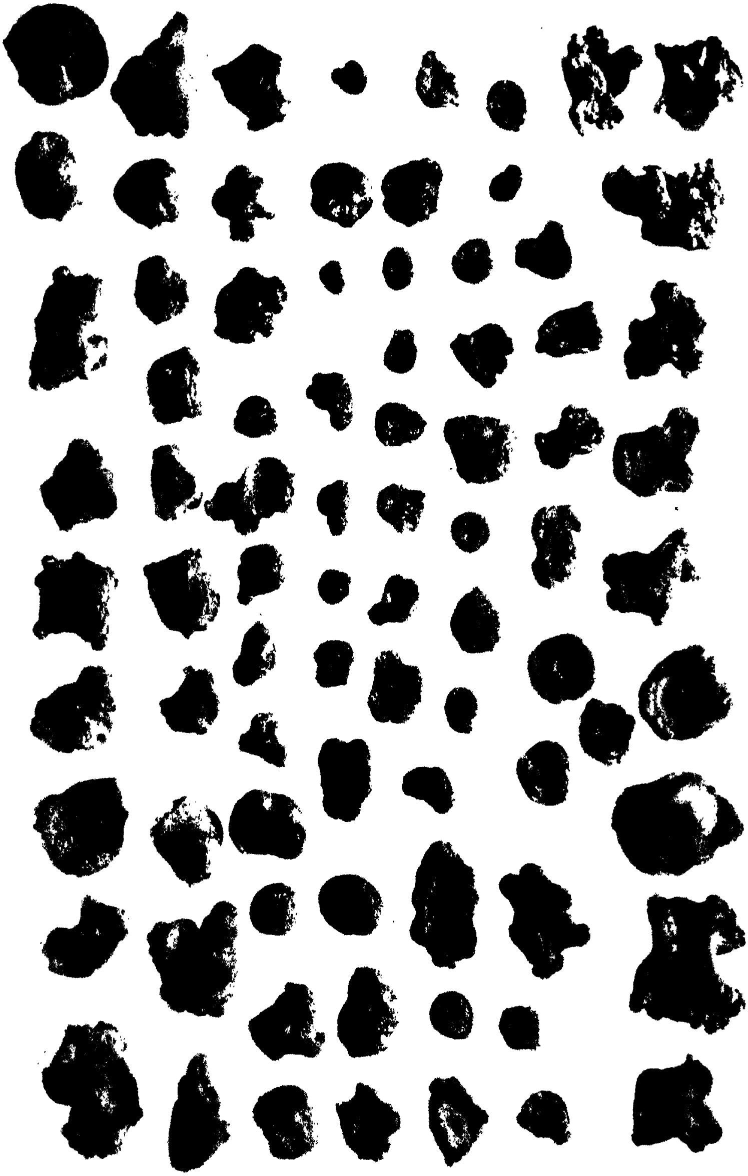
[3] SPESSARD, E. A., Prothallia of *Lycopodium* in America. BOT. GAZ. 63:362. 1918.



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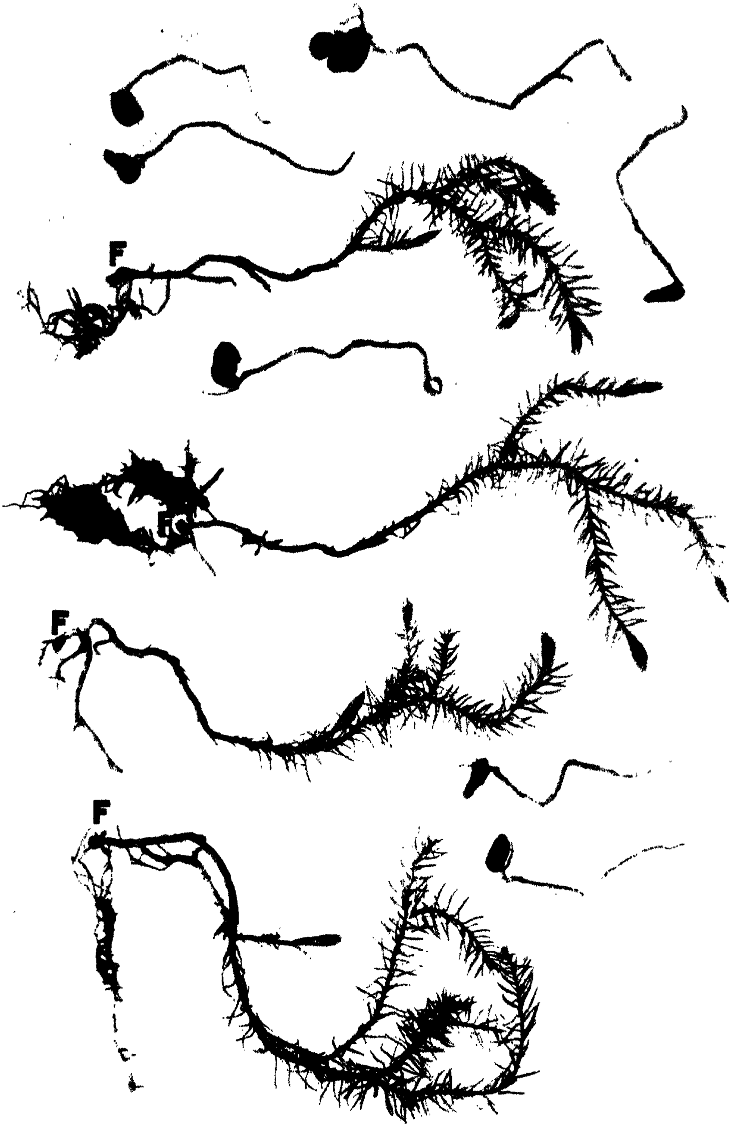

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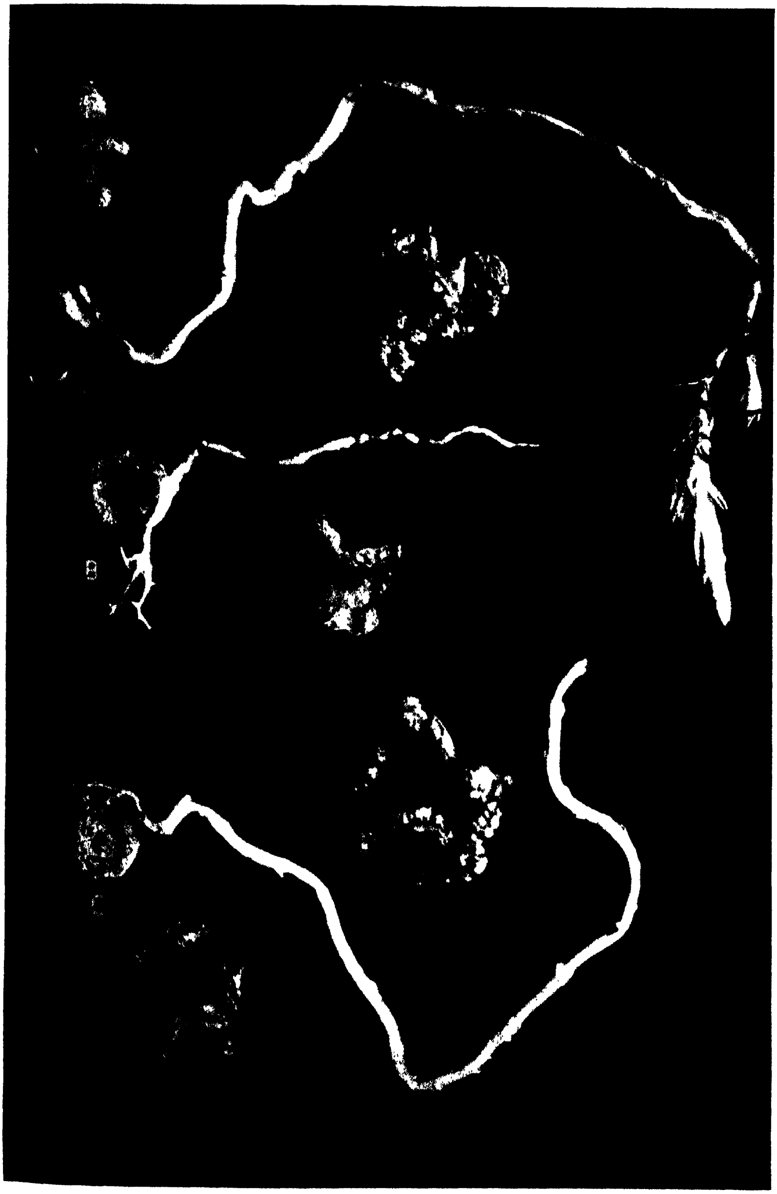

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The writer wishes to express his indebtedness to Dr. James B. Pollock, Exchange Professor from the University of Michigan, for invaluable suggestions and criticisms in the preparation of the manuscript. Thanks are due Dr. William H. Davis for aid in illustrating this paper.

Summary

1. The gametophytes of *L. complanatum*, *L. obscurum* var. *dendroideum*, and *L. clavatum* have been discovered in three different stations by the writer; the gametophyte of *L. obscurum* var. *dendroideum* has been discovered at another station by R. F. Martin. Several hundred prothallia of *L. obscurum* were discovered in a single station.

2. The commonest depth of growth for the gametophyte of *L. obscurum* is 2.5–7 cm.; the commonest diameter of the gametophyte without sporeling is 4–5 mm., and the largest diameter is 9 mm.; the commonest diameter with sporeling is 5–8 mm., the smallest is 4 mm., and the largest is 10 mm.

3. The gametophyte of *L. complanatum* can grow and produce a sporeling whether lying with the reproductive surface in a vertical position or in a horizontal position facing upward in the soil. The gametophyte of *L. obscurum* can likewise grow and produce a sporeling whether lying with the reproductive surface in a vertical or in a horizontal position. If in the latter, it may be facing either upward or downward in the soil.

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EXPLANATION OF PLATES XI–XIII

PLATE XI

L. obscurum: four rows on left show lower surface of prothallia; the rest show upper surface; × about 2.5.

PLATE XII

L. obscurum: four largest sporelings, from which prothallium has decayed, show foot (*F*); other sporelings are still attached to prothallium; slightly enlarged.

PLATE XIII

L. obscurum: *A*, sporeling developed from inverted prothallium that has decayed; *B*, sporeling developed from prothallium with reproductive surface standing vertically; *C*, sporeling developed from inverted prothallium; five prothallia with revolute margins; × about 2.5.

QUANTITATIVE ESTIMATION OF CHLORIDES AND SULPHATES IN EXPRESSED PLANT TISSUE FLUIDS[1]

ROSS AIKEN GORTNER AND WALTER F. HOFFMAN

In a comprehensive study of the chemical and physico-chemical properties of plant saps as related to their environment, the question often arises as to the relative importance of the organic and inorganic constituents of the sap. This is particularly true when plants growing on alkali soils are under investigation. HARRIS, GORTNER, HOFFMAN, and VALENTINE,[2] in studies carried out in the Tooele Valley of Utah during the summer of 1920, observed that the leaf tissue fluids of perennials of that region increased in osmotic concentration with the progress of the season, indicating a response on the part of the plant to progressive desiccation of the soil. They observed, however, that all of the physico-chemical properties of the saps did not change in the same ratios, and that some plants apparently increased their osmotic concentration by a marked accumulation of the inorganic constituents in the sap, while others appeared to hold the inorganic constituents practically constant and to hold in their leaves soluble carbohydrates. The former behavior is that of typical halophytes, while the latter is more or less characteristic of those plants which grow in soils having a low salt content.

The accumulation of solutes in a sap may reach exceedingly high osmotic concentrations. HARRIS, GORTNER, HOFFMAN, and VALENTINE[3] report values equivalent to 153 atmospheres in *Atriplex confertifolia*, and 169 atmospheres in *A. Nuttallii*, both typical halophytes. Inasmuch as many alkali soils contain chlorides and sulphates, it would appear desirable to estimate both of these ions in a study of the inorganic constituents of sap from plants growing upon such soils. It may be that halophytes differ among themselves in their ability to take up these ions, just as halophytes and non-halophytes differ in their accumulation of inorganic salts in general.

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[1] Published with the approval of the Director as Paper no. 370, Journal Series, Minnesota Agricultural Experiment Station.

[2] Unpublished.

[3] HARRIS, J. A., GORTNER, R. A., HOFFMAN, W. F., AND VALENTINE, A. T., Maximum values of osmotic concentration in plant tissue fluids. Proc. Soc. Exp. Biol. Med. 18:106–109. 1921.

Methods which have been suggested for the determination of chlorides and sulphates in plant materials do not well adapt themselves to a study of a large number of plant saps. The ideal method must meet the following conditions: (1) it must possess a high degree of accuracy; (2) it must require a minimum of material; (3) it must be rapid; and (4) it should not require complicated apparatus or exceptional skill on the part of the analyst. If possible it should be adapted to field laboratory conditions. During the past decade the biological chemists have made remarkable improvements in the methods for blood and urine analyses, all of which have been directed toward the simplification of procedure, use of small samples, and extreme accuracy. We believe, therefore, that some of these methods might advantageously be applied to plant saps, and the following methods have been found to fulfil all of the requirements.

Experimentation

DETERMINATION OF CHLORIDES.—The methods for the determination of chlorides in biological fluids are numerous. They fall roughly into two classes: (1) the destruction of the organic matter by an oxidizing agent such as nitric acid, or by charring and leaching of the chloride followed by complete ashing and then determining the chloride in the ash; and (2) the precipitation of the protein and the determination of the chloride in the filtrate.

Those falling in the first class are very time consuming, and in the case of oxidizing with nitric acid it is necessary to add an excess of silver nitrate before oxidizing to prevent a loss of chlorine. This is inconvenient, especially in the case of plant saps where the chloride content is not even approximately known, for in many cases it is necessary to "back titrate" a large excess of silver nitrate. In the case of ashing in a muffle, extreme precautions must be taken to prevent loss of chlorides.

The determinations depending upon the precipitation of the proteins are much simpler and less time consuming. There are many methods for precipitating the proteins, but the method used by WETMORE[4] for the determination of chlorides in blood appeared to be the most applicable to plant sap. In this method the proteins are precipitated by copper hydroxide, as described by HARDING and MASON,[5] and the chlorine determined by the procedure used by RAPPLEYE.[6] This method, with slight modifications, was used for determining chlorides in plant sap. The procedure adopted is as follows: 10 cc. of plant sap is placed in a 50 cc. volumetric flask, 5 cc. of 8 per cent copper sulphate, 3 cc. of a normal solution of sodium hydroxide, and enough water to make a total volume (in the flask) of about 35 cc. are added. The proteins are coagulated by heating the flask in a boiling water bath for one minute. The flask is shaken to insure complete precipitation of the protein. The flask and contents are cooled in running water, made to volume, and thoroughly mixed. The precipitate is separated by centrifuging, and the clear liquid is then decanted into a dry centrifuge tube containing 1 gm. of dry calcium hydroxide, thoroughly mixed, and again centrifuged. The treatment with calcium hydroxide serves to remove any coloring matter or excess of copper hydroxide. The chlorine is then determined by pipetting 10–20 cc. of the clear supernatant liquid into a porcelain casserole, adding 5 cc. of concentrated nitric acid, an excess of standard silver nitrate, and stirring until the silver chloride separates. Two cc. of ferric alum solution is then added, and the excess of silver nitrate titrated with standard potassium thiocyanate solution. The first reddish tinge is the end point.

To calculate the amount of chlorine, subtract one-half of the volume of potassium thiocyanate used from the number of cubic centimeters of silver nitrate added, and multiply this by the aliquot used for the titration. The number of cubic centimeters of silver nitrate used multiplied by 0.004 gives grams of chlorine in a 10 cc. sample of sap. The reagents required are: (1) 8 per cent copper sulphate solution; (2) a normal solution of sodium hydroxide; (3) calcium hydroxide powder; (4) concentrated nitric acid; (5) a 10 per cent solution of ferric alum; (6) standard silver nitrate, made by dissolving approximately 20 gm. of silver nitrate in one liter of water and diluting until 1 cc. is equivalent to 1 cc. of potassium chloride containing 0.004 gm. chlorine per cc.: (7) potassium thiocyanate solution, made by dissolving approximately 5.5 gm. potassium thiocyanate in one liter of water and diluting until 2 cc. are equivalent to 1cc. of silver nitrate solution.

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[4] WETMORE, A. S., Determination of chlorides in blood. Jour. Biol. Chem. 45:113–118. 1920.

[5] HARDING, V. J., AND MASON, E. H., The estimation of chlorides in body fluids. Jour. Biol. Chem. 31:55–58.

[6] RAPPLEYE, W. C., A simple application of the Volhard principle for blood plasma chlorides. Jour. Biol. Chem. 35:509–512. 1918.

It is necessary to determine the amount of chlorine obtained on a "blank," using only the reagents. If this is significant, it must be subtracted from the total chloride content. Most of the "analytical" reagents on the market are sufficiently pure to use without making this correction. By this method good checks were obtained on duplicate determinations of chlorine in the centrifuged sap of squash leaves. Sodium chloride was quantitatively recovered after its addition to the sap, as shown in table I.

TABLE I

GRAMS OF CHLORINE RECOVERED FROM 10 CC. SQUASH SAP TO WHICH VARYING AMOUNTS OF SODIUM CHLORIDE HAD BEEN ADDED

Chlorine added	Chlorine recovered	Theoretical chlorine present	Difference	Chlorine recovered per liter	Theoretical chlorine per liter of sap
None	0.0064			0.64	
0.0059	0.0123	0.0123	0.0000	1.23	1.23
0.0118	0.0187	0.0182	+0.0005	1.87	1.82
0.0235	0.0291	0.0299	−0.0008	2.91	2.99
0.0471	0.0538	0.0535	+0.0003	5.38	5.35
0.0706	0.0761	0.0770	−0.0009	7.61	7.70
0.0942	0.1010	0.1006	+0.0004	10.10	10.06
0.1177	0.1228	0.1241	−0.0013	12.28	12.41
0.2943	0.3019	0.3007	+0.0012	30.19	30.07
0.3536	0.3591	0.3600	−0.0009	35.91	36.00

We recently had occasion to determine the amount of chlorine present in a sample of sap obtained from the leaves of *Atriplex Nuttallii.* Using this method we found 84.23 gm. of chlorine per liter. A sample of the sap was then dried and carefully ignited in platinum in a muffle, at a temperature not exceeding 500° C.

A light gray ash, free from carbon, was obtained. A chlorine determination on this ash indicated 83.52 gm. of chlorine per liter of sap, or 99.15 per cent of the chlorine indicated by the proposed method. This is regarded as proof that all of the chlorine is accounted for in the proposed method, for the actual figures obtained (2.5 cc. of sap were used in the actual titration) were 0.2105 gm. chlorine by the proposed method and 0.2088 gm. chlorine in the ash. The difference between these values is well within the experimental error.

This method is simple and takes but a short time to complete. With sufficient apparatus, a large number of determinations can be made in a day. The method is admirably adapted for carrying out the chloride determinations in a field laboratory. If it is impractical to do this, the samples may be preserved by pipetting portions of the sap into heavy walled tubes, adding a drop of formaldehyde, and sealing off the tubes with a flame.[7] Samples prepared in this manner may be preserved indefinitely. A satisfactory method of opening such sealed tubes is to heat the tube by looping around it near the top a piece of high resistance (Nichrom no. 30) wire, which is then heated red hot by an electric current controlled by a rheostat. After the tube becomes hot, the wire is removed and a drop of water placed on the heated circle on the glass, which produces a crack in the tube so that the top can easily be removed. The contents of the tube is then poured out and the tube washed until all of the contents are removed.

DETERMINATION OF SULPHATES.—The methods employed for the determination of sulphates, including unoxidized sulphur, are all based on the destruction of the organic matter and precipitation of the sulphate as barium sulphate. They depend upon the destruction of the organic matter by oxidation, using sodium peroxide, nitric acid, or some similar oxidizing agent. These methods are either disagreeable or very time consuming.

For the determination of the sulphate in plant sap, it was thought that the BENEDICT-DENIS method[8] as used by the biological chemists for determining sulphur in urine might satisfactorily be used. The method is simple, rapid, and does not require elaborate apparatus or special technique. The procedure as we have used it is as follows: 5–10 cc. of centrifuged or filtered sap is placed in an 11.5 cm. porcelain evaporating dish. Ten cc. of the BENEDICT-DENIS reagent,[9] which is ample for 10 cc. of sap, is added to the contents of the evaporating dish. This mixture is then evaporated to approximate dryness on a water bath,[10] and then carefully ignited, at first over a small flame and finally to dull redness for a few minutes, thus destroying the last traces of organic matter. After the dish cools, the ignition residue is dissolved with the aid of heat in dilute hydrochoric acid. The sulphate is then precipitated and weighed as barium sulphate. It is necessary to make a "blank" determination of the reagent and to subtract this from the total weight of barium sulphate. By using high grade chemicals a blank of 2.8–3.5 mg. of $BaSO_4$ for 10 cc. of the reagents is obtained. It is desirable not to have a blank of more than 4.0 mg. for 10 cc. of the reagent, especially when the determinations are to be made on a small amount of sap containing but little sulphate.

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[7] This method of preserving samples of sap is not original with us; Dr. J. ARTHUR HARRIS has employed it for several years.

[8] BENEDICT, S. R., The estimation of total sulphur in urine. Jour. Biol. Chem. 6:363–371. 1909. DENIS, W., The determination of total sulphur in urine. Jour. Biol. Chem. 8:401–403. 1910.

For the most satisfactory results the amount of barium sulphate obtained should be about 0.2 gm., but good results have been obtained when the amount was as low as 0.025 gm. and as high as 1.0 gm. These values cover a range of approximately 1 gm. to 40 gm. SO_4 per liter of sap. When the amount of sulphate is above 15–20 gm. per liter, 2–5 cc. of the sap would be sufficient for a very accurate determination. The difference between duplicate determinations is very small, usually not over 1 or 2 mg. $BaSO_4$, which is less than 0.1 gm. of sulphate per liter.

To test the accuracy of this method, a series of determinations was made on centrifuged cabbage sap. The sulphate was determined in 10 cc. of sap, and 10 cc. of sap plus varying amounts of potassium sulphate. The results of these determinations, given in table II, show that the method is accurate for plant sap. The rather large values for sulphate in the original cabbage sap probably are not due to inorganic sulphates, but rather to organic sulphur compounds such as mustard oils and sulphur containing proteins. It is well known that volatile oils of the Cruciferae contain organic sulphur. Such sulphur would be oxidized to sulphate by this procedure, and it would be impossible to distinguish between organic and inorganic sulphur by the proposed method. We believe, however, that the amount of organic sulphur will always be low in plant saps (with the possible exception of those plants having sulphur compounds in their volatile oils), and need not seriously affect studies of the inorganic sulphate content of the saps. Even if organic sulphur is present in the volatile oils, the apparent sulphate content due to such sulphur will probably never exceed 5 gm. per liter of sap.

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[9] This reagent is made by dissolving 25 gm. of crystalline copper nitrate, 25 gm. of sodium chloride, and 10 gm. of ammonium nitrate in enough water to make 100 cc. of solution.

[10] If the dishes are removed from the water bath before completely dry and the ignition carried out within a short time, very little if any spattering occurs.

TABLE II

GRAMS OF SULPHATE RECOVERED FROM 10 CC. CABBAGE SAP TO WHICH VARYING AMOUNTS OF SULPHATE HAD BEEN ADDED

SO_4 added	SO_4 recovered per 10 cc.	Theoretical per 10 cc.	Difference	SO_4 recovered per liter	Theoretical per liter
None........	0.0336	....	....	3.36	....
None........	0.0333	....	....	3.33	....
0.02.........	0.0542	0.0535	+0.0007	5.42	5.35
0.05.........	0.0835	0.0835	0.0000	8.35	8.35
0.10.........	0.1340	0.1335	+0.0005	13.40	13.35
0.15.........	0.1833	0.1835	−0.0002	18.33	18.35
0.20.........	0.2350	0.2335	+0.0015	23.50	23.35
0.30.........	0.3332	0.3335	−0.0002	33.32	33.35

Summary

Methods have been described for the rapid and accurate determinations of chlorine and sulphates in plant saps. These methods are essentially as described by WETMORE for chlorides in blood and by BENEDICT for sulphur in urine. The manipulations are simple, and neither elaborate apparatus nor exceptional skill is required.

UNIVERSITY FARM
ST. PAUL, MINN.

RELATION OF KIND OF FOOD RESERVES TO REGENERATION IN TOMATO PLANTS

MARY ELIZABETH REID

(WITH TWO FIGURES)

A series of experiments is being carried on with tomato cuttings, to determine the relation between the character of the reserve foods which they contain and the quantity and quality of the growth response when grown under different environmental conditions, such as variations in nutrient solutions and exposure to light. Plants of two different types have been produced, according to the method suggested by KRAUS and KRAYBILL[1] in their work on vegetation and reproduction in the tomato. One type of plant, high in carbohydrates and low in nitrates, was obtained by first growing the plants to a height of 7–8 inches, and then transferring them to a nitrogen-free sand medium to which a nutrient solution lacking nitrogen was applied. The other type, low in reserve carbohydrates such as sugars and starches and high in nitrates, was obtained by growing the plants continuously in a soil rich in nitrogenous content. Exact quantitative determinations of the food reserves in the two types of plants used in these experiments have not been made up to the present time. Microchemical tests, however, indicate practically the same distribution of substances as KRAUS and KRAYBILL found in the plants with which they worked. Since they found a gradient in carbohydrate content of tomato plants increasing from the apex to the base, and a gradient in nitrate content increasing from the base to the apex, it was thought desirable to cut the longer defoliated stems into three portions, base, middle, and apex, thus by the use of these segments obtaining more closely limited relations between the nitrogenous and carbohydrate content of the stem.

Each cutting was weighed and placed in a 250 cc. test tube one inch in diameter. Two nutrient solutions (modifications of Knop's solution) were used, one containing nitrate nitrogen and the other without nitrogen in any form. The two solutions contained approximately equal amounts of potassium, sulphur, magnesium, phosphorus, calcium, and chlorine. Nitrogen was present as an additional element in one of the solutions. These solutions were diluted with distilled water to such an extent that the total concentration of salts was 0.25 per cent in the solution containing nitrogen and 0.19 per cent in the solution without nitrogen. The solution for each cutting was replaced every four days. The original plan was to allow the cuttings to remain in the nutrient solution until the food reserves were exhausted. It was found that, in tests in which cuttings high in carbohydrates were used, from four to five weeks was the average time necessary for the fullest utilization of the food reserves present in the cuttings. In the case of the nitrogen-high cuttings it was found that the growing period was never longer than from one to two weeks for cuttings kept in darkness. If the cuttings were kept at ordinary room temperature longer than the growing period, decay set in. Since the time for the utilization of the food reserves, as evidenced by growth, was quite different for cuttings from the two types of plants, it seemed advisable to allow those from carbohydrate-high and those from nitrogen-high groups time for the fullest utilization of such reserves.

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[1] KRAUS, E. J., and KRAYBILL, H. R., Vegetation and reproduction with special reference to the tomato. Oregon Agric. Coll. Exp. Sta. Bull. 149. 1918.

Two sets of experiments were conducted, one in the light and the other in darkness. These were repeated three different times, always with the same general results. The quantity and character of growth of the cuttings from the two types of plants were very different under the same external conditions of light, temperature, and nutrient solution. To illustrate the differences in growth obtained with cuttings of different composition, the growth responses of the basal cuttings under different conditions will be described somewhat in detail.

Experiments

BASAL CUTTINGS GROWN IN LIGHT AND IN SOLUTIONS CONTAINING NITRATES.—*Behavior of cuttings from carbohydrate-high plants* (fig. 1*A*).—These cuttings produced both roots and shoots very abundantly. Nearly all the buds which were present when the cuttings were made developed into shoots. Even below the level of the nutrient solution, and in the region of greatest root development, there was considerable growth of the buds. The leaves were

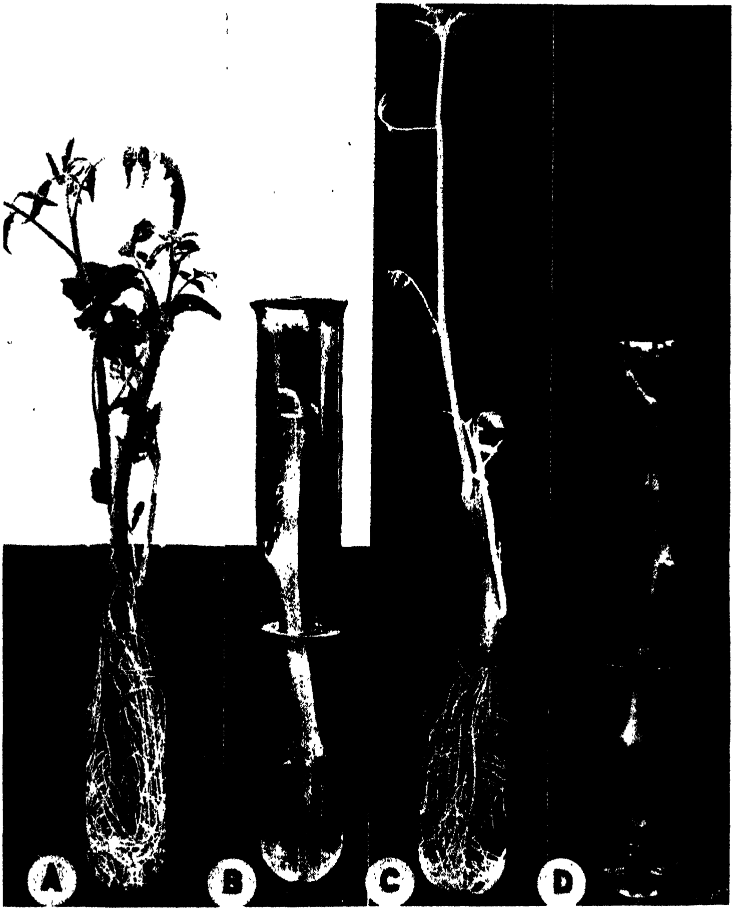

FIG. 1.—Basal cuttings grown with nitrates in nutrient solution: *A*, carbohydrate-high cuttings grown in light; *B*, nitrogen-high cuttings grown in light; *C*, carbohydrate-high cuttings grown in darkness; *D*, nitrogen-high cuttings grown in darkness.

large. The roots were very numerous, but were often without secondary rootlets. Each cutting became conspicuously greener during the time it was in the nutrient solution.

Behavior of cuttings from nitrogen-high and carbohydrate-low plants (fig. 1*B*).—In most cases these cuttings produced no roots whatever, but in case the roots did develop, they did not attain a length of more than three-fourths of an inch and had a dense growth of root hairs. In one lot of cuttings shoots developed at most of the nodes, but such shoots remained small and the leaves on them were abortive. In this particular lot of material the carbohydrate reserves were unusually low. In the other two lots, in which microchemical tests indicated a somewhat greater carbohydrate reserve, the shoots grew longer, and developed full sized leaves, which were very soft in texture and of a uniform green color.

BASAL CUTTINGS GROWN IN DARKNESS AND IN SOLUTIONS CONTAINING NITRATES.—*Behavior of cuttings from carbohydrate-high plants* (fig. 1*C*).—The general growth responses were very like those described for similar cuttings grown in the light, except that in the dark there was produced scarcely half the total quantity of both shoots and roots produced in the light. There was also a marked difference in the distribution of shoots which grew on these cuttings as compared with that of the corresponding lot grown in the light. In the light all the buds present developed into shoots, whereas in the dark in most cases the topmost bud only developed. The shoots produced in darkness were markedly etiolated. In some cases the individual shoots attained a length of fifteen inches, whereas six inches was the maximum length attained by shoots grown in the light. The roots developed to about the same length under the two sets of conditions. Preliminary microchemical tests (made only in the last experiment) indicated the presence of a very small amount of nitrate in the basal portions of some of the young shoots developed in darkness, but no nitrates were found at their apices. In most cases no nitrates could be detected in the old cuttings at the end of the experiment, but some starch was still present. The amount of starch was roughly estimated as about two-thirds the quantity originally present.

Behavior of cuttings from nitrogen-high and carbohydrate-low plants (fig. 1*D*).—There was no growth of either shoots or roots from such cuttings. The cuttings were very susceptible to bacterial decay. Small portions of the petiole that remained attached to the cuttings showed softening and decomposition within a very few days.

BASAL CUTTINGS GROWN IN LIGHT AND IN SOLUTIONS WITHOUT NITRATES.—*Behavior of cuttings from carbohydrate-high plants.*—(fig. 2*A*.) No shoots were produced by such cuttings, but there was in most cases an abundant root growth. Microchemical tests of the old cuttings made at the end of the growth period showed a great abundance of starch still present. Although no nitrates could be detected in these cuttings, when originally placed in the nitrate-free nutrient solution, yet after root growth had proceeded for some time, nitrates could be detected. Nitrates were found to be present in greater abundance at the bases (in the region of root growth) than at the apices of the cuttings.

Behavior of cuttings from nitrogen-high and carbohydrate-low plants (fig. 2*B*).—In one experiment some of the cuttings produced a few short, very coarse, and somewhat branched roots, while in another lot of cuttings, in which the carbohydrate reserves were lower, no roots were produced. In an experiment with the cuttings containing the least amount of carbohydrates all the buds grew to some extent, but their development was slight and in no case did the leaves fully expand. On the other hand, the cuttings containing more carbohydrates produced a greater growth of shoots, with fully developed leaves of a uniform green. The roots developed after the growth of shoots was well started. This growth of roots may have been possible because carbohydrates were synthesized by the leaves.

BASAL CUTTINGS GROWN IN DARKNESS AND IN SOLUTIONS WITHOUT NITRATES.—*Behavior of cuttings from carbohydrate-high plants* (fig. 2*C*).—The type of growth of such cuttings was practically the same as that in the corresponding lot grown in the light. The cuttings produced numerous roots but no shoots. Only about half the quantity of roots was produced in darkness as in the light. Microchemical tests showed large quantities of starch still present in the cuttings when these were removed at the end of the experiment. It was roughly estimated that about three-fourths of the starch originally present was still in the cuttings. Nitrates were found to be present, as in the case of similar cuttings grown in the light and in like nutrient solutions.

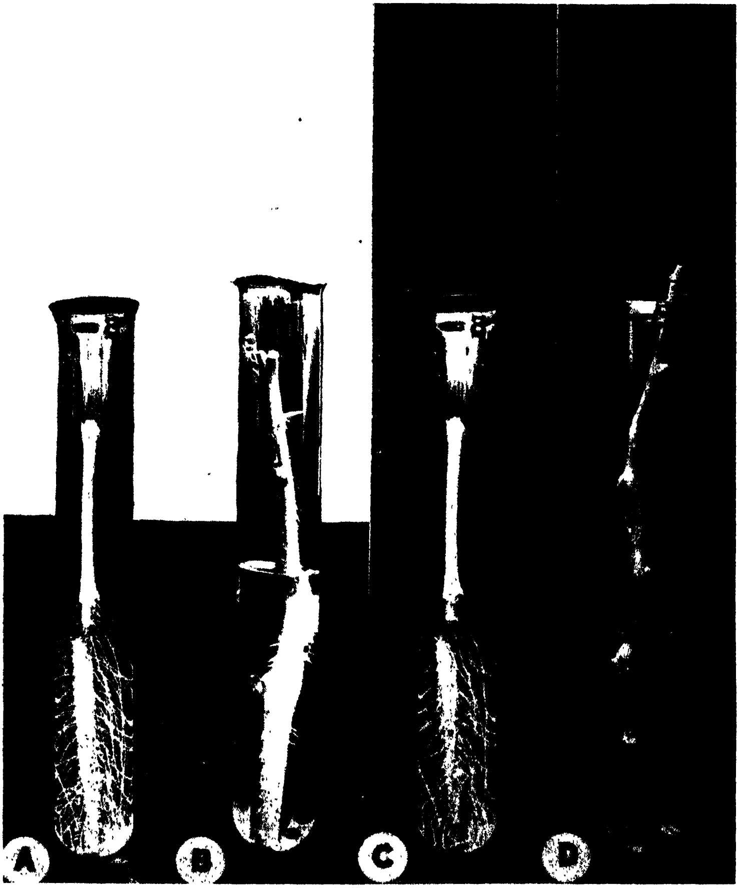

FIG. 2.—Basal cuttings grown without nitrates in nutrient solution: *A*, carbohydrate-high cuttings grown in light; *B*, nitrogen-high cuttings grown in light; *C* carbohydrate-high cuttings grown in darkness; *D*, nitrogen-high cuttings grown in darkness.

Behavior of cuttings from nitrogen-high and carbohydrate-low plants (fig. 2*D*).—In one lot of material, in which there was a small amount of reserve carbohydrates in the cuttings, there was a very small amount of shoot growth but no root growth. In another lot of cuttings, in which the carbohydrate reserve was still lower, there was no production of either shoots or roots.

BASAL, MIDDLE, AND TOP CUTTINGS.—In the case of carbohydrate-high cuttings grown in the light without nitrates, in the nutrient solution the middle and top portions produced some shoot growth, whereas no growth of shoots occurred from the basal portions. In most experiments in which such cuttings were grown with nitrates in the nutrient solution, the total growth of roots and shoots was greater from the basal than from the middle portions, and greater from the middle than from the top portions. It should be stated in this connection that the basal cuttings had a greater average weight than the middle cuttings, and the middle cuttings a greater average weight than the top cuttings.

The vegetative cuttings, both in the solution containing nitrates and in the one without it, showed slight variations in the relative amounts of shoot and root growth by the different segments. In the cases where the relative differences between carbohydrate and nitrogen reserves in the different levels of the stem may be very slight, further experimentation will be carried on to determine more definitely the relationships of root and shoot growth.

In the case of carbohydrate-high cuttings grown in the solution containing nitrates, it was generally observed that when equal weights of cuttings from the three regions of the stems were compared, the greatest amount of shoot growth was made by the apical cuttings, whereas the basal portions produced the least shoot growth. In the case of root growth it was the basal portions which produced the greatest amount, both in number and weight of roots. The middle portions produced roots second in number and weight, and the top portions the least amount.

The roots which appeared on the middle and top cuttings in most cases were confined to a limited region around the lowest node. The roots from the middle portions were longer and more branched than those produced by the top or basal portions. In most cases the roots from cuttings grown in the nutrient solution without nitrogen remained in a healthy condition for a longer time than those grown in the solution containing nitrates. The latter frequently showed a brownish discoloration.

In the carbohydrate-high cuttings grown in the nutrient solution without nitrogen, the leaves were small, pale green, and relatively stiff in texture. In this case the nitrogen supply was limited, both within and without the plant. When there was a great abundance of nitrogen both in the plant and in the solution, but with a low carbohydrate reserve in the cuttings, the leaves were soft in texture.

Summary

1. It was very noticeable that in cuttings high in nitrogen, grown in the light in a nutrient solution lacking nitrates, considerable shoot growth resulted, and that in some cases more growth resulted than if nitrates were present in the solution.

2. The presence of nitrates in the nutrient solution in the case of nitrogen-high cuttings appeared to be unfavorable for root growth. If the carbohydrate reserves are exceedingly limited, the nutrient solution containing nitrates may also be unfavorable for shoot growth. In striking contrast with this condition, the same sort of solution favored development of shoots from cuttings high in carbohydrates. In this case root growth was also favored, although to a less extent.

3. In general, it seems that when the carbohydrate reserve is high and the nitrogen supply within the plant or in the nutrient solution is low, there may be a vigorous root growth. When the relative percentage of nitrogen either within or without is slightly higher, there may also be a vigorous shoot growth.

These investigations were carried on in the department of plant physiology at the University of Wisconsin, and helpful directions and criticisms were given by those in charge.

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AN UNUSUAL GROWTH OF MOLD[1]

FREDA M. BACHMANN

(WITH THREE FIGURES)

A few years ago (1917) a very unusual growth of mold was found, which had formed in a bottle of grapejuice. The grapejuice had been boiled. The bottle was filled to the top of the neck with the hot liquid and immediately covered with thin cloth and sealed with sealing wax. It was stored in a cellar in which the temperature varied from about 60°F. in summer to nearly freezing in winter. The age of the culture is uncertain, but the grapejuice was bottled before the year 1900, so that when it was found, it was at least seventeen years old. The 8 ounce bottle was 9 inches high, 3. inches wide, and 1.25 inches thick. The cylindrical neck measured 3 inches in length and somewhat less than 1 inch in 'diameter. When I first saw it, the liquid and the mold filled the bottle to within an inch of the top (fig. 1).

Through handling, the growth became loosened from one side of the neck. Although the seal seemed perfect, a small amount of air must have entered, enough to support a slow vegetative growth of the mold. The sealing wax was very brittle, and it may be that with different changes in temperature it had a greater viscosity at times and at others more brittleness, resulting in some slight cracks.

Since the growth of the mold took place only at the surface of the liquid, the resulting mass of hyphae was shaped by the neck of the bottle into a firm cylindrical mass and forced downward. As this gradually lengthened, it must have become longer than the bottle, and, because of the narrowness of the bottle, the mold growth formed a number of folds like a letter *S*. The absence of any growth of the hyphae from the surface of the mass submerged in the liquid is a striking example of the dependence for growth of molds upon the presence of atmospheric oxygen.

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[1] Published by permission of Director of Wisconsin Agricultural Experiment Station.

There was no evident change in the appearance of the growth from 1917 until about a year ago, when I decided to open the bottle and test the hyphae for viability. The seal was broken with aseptic precautions and replaced by a sterile cotton plug. Microscopic examination of the mycelium showed it to be septate. Some of the cells were without content, apparently dead cells, in others the scanty protoplasm filled only part of the cell, while in still others the microscopic picture was that of slightly plasmolyzed or even normal cells. The ends of some of the hyphae were much enlarged. Fig. 2 shows parts of a number of hyphae as they were when the bottle was first opened.

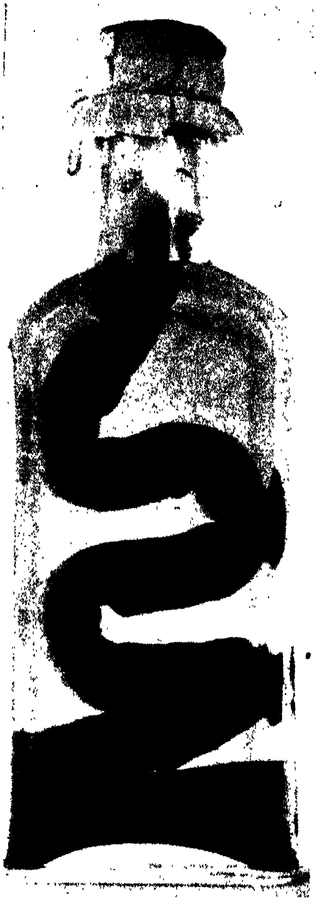

FIG. 1

A transfer of some of the surface hyphae was made to sterile potato agar. A gray-green growth resulted in a few days. The mold sporulated readily on this medium. Fig. 3 represents the conidiophores. The mold appears to be a species of *Aspergillus*. Since the seal was broken the mold has grown considerably, but it is no longer in the neck of the bottle. The liquid has evaporated, so that it is about two-thirds of an inch below the neck. The mold growth is spread out on the surface and is a much softer, looser growth. Spores have been formed in abundance.

Since it is not known how long a time was required to produce such a growth of mold, it seemed of interest to attempt to reproduce it. In March 1922, three bottles of a liquid consisting of grapejuice diluted with a 3 per cent dextrose solution were heated in the steamer for 30 minutes. As soon as the liquid was cool enough it was inoculated with mold spores. One bottle was inoculated with a species of

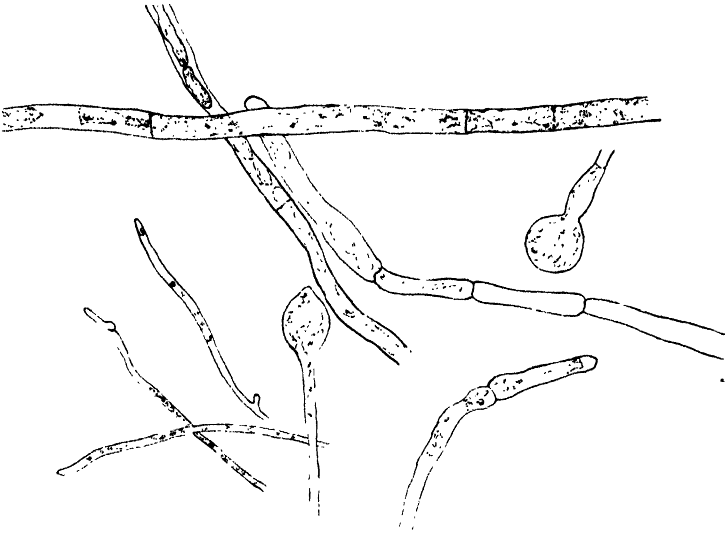

Fig. 2

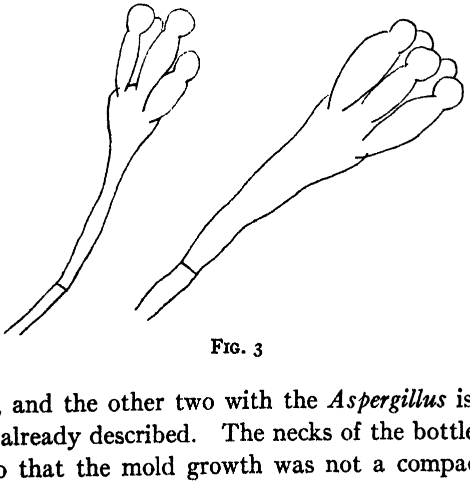

Fig. 3

Penicillium, and the other two with the *Aspergillus* isolated from the growth already described. The necks of the bottles used were not long, so that the mold growth was not a compact, perfectly cylindrical mass, but it was shaped somewhat by the neck of the bottle. The *Aspergillus* produced growths 1 inch long, and *Penicillium* a growth nearly 3 inches long. The growth of the mold in the bottle shown in fig. 1 measures at least 14 inches in length. It did not seem to change after it was found in 1917, so that probably nearly all of this growth was before that time. The rate of growth must be influenced by the amount of oxygen present, and since it is not possible to compare the porosity of the seal of the original with that of the bottles prepared last March, no conclusions can be drawn as to the age of the culture.

If the seal had been very imperfect, there would have been considerable loss in the liquid, due to evaporation. From the compactness of the mass before the seal was broken, and the evidence showing the seal to have permitted very little passage of air, it appears that the growth must be the result of many years at least.

AGRICULTURAL COLLEGE
MADISON, WIS.

BRIEFER ARTICLES

EDWARD W. D. HOLWAY

(WITH PORTRAIT)

EDWARD WILLET DORLAND HOLWAY died in Phoenix, Arizona, March 31, 1923, at the age of seventy years. He was born in Adrian, Michigan, in 1853, and when about one year old his parents moved to what were then the frontier settlements of northeastern Iowa. His boyhood years were spent on a farm near the village of Hesper. As a youth he was preparing himself for the profession of civil engineering, but was interrupted by severe illness. During his convalescence he accepted a position with the Winnesheik County Bank of Decorah, Iowa, an institution with which he remained connected for thirty-five years.

During his long and successful career as a banker he became interested in botany, eventually specializing in the Uredineae, and while still engaged in the banking business he built up a very extensive collection and valuable library along his chosen field. Wishing to devote all his time to botanical research and traveling, in 1904 he retired from active connection with the bank, and moved to Minneapolis, in order to be in closer touch with academic life. Here he presented his collections and library to the University of Minnesota, and accepted the position of Assistant Professor of Botany, which he held until his death.

Mr. HOLWAY published many papers in his chosen field, both individually and in collaboration with J. C. ARTHUR and others. His greatest work is a monographic publication on the North American Uredineae. The first part of this appeared in 1905, and at the time of his death he had published four parts and left the completed manuscript for the fifth part, all devoted to the genus *Puccinia*.

Mr. HOLWAY was a great traveler, and not the least valuable of his scientific contributions are his collections of fungi and other plants from various little known parts of the world. While still in the banking business he traveled extensively in the United States and Mexico, and after his retirement he made several trips to Central America and western Canada, besides two long journeys in South America, the first covering the western part of that continent from southern Chile to Ecuador, the second largely confined to eastern and southern Brazil. He had just returned from the last journey when he was attacked by the illness which terminated in his death.

A man of indomitable energy, he was always fascinated by exploring little known and inaccessible regions. When nearly fifty years of age he became an enthusiastic alpinist and thoroughly mastered the art of mountain climbing. He undertook a number of difficult exploring and climbing expeditions, particularly in the Canadian Rockies, the Caribou Range, and the Selkirks. In the latter range alone he climbed twenty-three summits over 10,000 feet high, thirteen of them first ascents and nine of these without guides.

At the time of his death Mr. HOLWAY was a member of the Royal Geographical Society, the American Alpine Club, the Canadian Alpine Club, and the Society of Sigma Xi. At one time he was a member of the Botanical Society of America. He was a remarkable example of a successful business man with an essentially scientific mind, who finally turned completely away from commercial affairs to devote all his time to what he considered the more worth while pursuit of science.—F. K. BUTTERS, *University of Minnesota, Minneapolis, Minn.*

CURRENT LITERATURE

NOTES FOR STUDENTS

Soil acidity and plant distribution.—Abstracts of a number of papers by WHERRY,[1] dealing with the problems of acidity of soils and the relation of acidity to plant distribution, are brought together and summarized in this report. Indicators to be used, methods of making determinations and for tabulation of data are suggested. Studies on ferns, orchids, and ericads are reported, with the range of acidity or alkalinity for each species, and the most frequently found, or optimum, value. It is claimed that the peculiarities of the flora of the New Jersey pine barrens, as well as certain similarities between peat bog and salt marsh border floras, can be explained on the basis of differences in soil acidity.

In a lengthy paper OLSEN[2] reports the results of studies with meadow and woodland plants on Danish soils. Acidity of the soils, as determined by an improved colorometric method, runs from P_H 3.4 to P_H 8. Within the P_H range for any given species there is a narrower range in which the species has its maximum frequency. Pot and solution culture experiments confirmed field observations, in that acid soil plants became chlorotic and were unable to make good growth in neutral or slightly acid media, while alkaline soil plants were at a decided disadvantage in acid media. Attempts were made also to study the effects of aluminum and of nitrate nitrogen as compared with ammonia nitrogen on soil acidity and plant distribution and growth. Both factors are considered to be without effect, but the experiments on which the conclusions are based are far from convincing to anyone reasonably familiar with the literature in these fields.

Both authors believe that the distribution of plant species under natural conditions varies regularly with the soil reaction. That plants differ in acid or alkali tolerance is unquestioned. That there is also an intermediate range of soil reaction, within which each species is able to make good growth when other conditions are likewise favorable, is probable. When, however, one considers the great variability in soil reaction, vertically and horizontally, met in the field, and the enormous number of physical, chemical, and biological factors involved in the growth of any individual plant, one wonders whether the

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[1] WHERRY, E. T., Soil acidity: its nature, measurement, and relation to plant distribution. Smithsonian Rept. 247–268. 1923.

[2] OLSEN, CARSTEN, Studies on the hydrogen ion concentration of the soil and its significance to the vegetation, especially to the natural distribution of plants. Compt. Rend. Trav. Lab. Carlsberg **15**: 1–166. 1923.

"hydrogen ion" is not being called upon to explain too much. Frequently the so-called acid plants are found growing vigorously in non-acid habitats.

It is true that each plant is a measure of the conditions, internal and external, under which it grows; but its growth is the resultant of many factors, few of which are overwhelmingly dominant. If any factor stands out clearly in world distribution of vegetation, it is water supply, not hydrogen ion concentration of soil waters. If one is trying to set sharp limitations to classes of plants as related to soil acidity, perhaps a more accurate method would be desirable. SALTER and MORGAN[3] have shown that the soil-water ratio affects the P_H value, and other factors may also affect the values obtained, as the amount of indicator used, the amount of extract tested at a time, the length of time the chemicals interact, the degree of turbidity of the soil extract, and the quality of the color chart used. Unless these are carefully standardized, potentiometric checks upon readings would be very desirable. Finally, how are we to classify plants which are sometimes found growing in soils that vary vertically by considerable P_H values? Is a plant growing on a neutral soil, with good root development extending down into an acid subsoil, to be classified as belonging to an acid habitat? When one finds the same species growing a hundred yards away on an acid surface soil with neutral or alkaline subsoil, and good root development in both, how does one arrive at a classification, or can the investigator take his choice?—R. B. DUSTMAN.

Enzymes and fungal infection.—Several years ago the discovery was made that mold spores contain enzymes. This has stimulated further research on the enzyme equipment of spores, and the possible influence of this equipment on the infection of host plants. HARTER and WEIMER[4] have demonstrated the occurrence of amylase and pectinase[5] in the spores of *Rhizopus tritici* and *R. nigricans*, regardless of the temperature at which the spores had been produced. The pectinase was used with sweet potato tissue and found to attack the middle lamella. The enzyme is more active, or more abundant in the spores of *R. tritici* than in *R. nigricans*.

Since various species of *Rhizopus* differ as to temperature requirements, the effects of temperature on pectinase production by the mycelium have been investigated[6] for nine parasitic and two non-parasitic species. The two non-parasitic species, *R. microsporus* and *R. chinensis*, both produce more pectinase than *R. nigricans*, a parasitic form. The production of pectinase is least at high temperature, 40° C., and increases with decrease of temperature to 9° C. The cell walls of old potatoes are more readily attacked than those of freshly dug new potatoes. These fungi, however, cannot penetrate the unbroken skin of the sweet potato. Infection[7] occurs through wounds, apparently only when some dead tissue gives the fungus a saprophytic start. With this start, pectinase is developed, which dissolves the middle lamella and causes cells to die, thus extending the necrotic region. Since wounding is a preliminary necessity to *Rhizopus* rot in the sweet potato, losses can largely be obviated by care to avoid injury in digging, storing, and marketing.—C. A. SHULL.

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[3] SALTER, R. M., and MORGAN, M. F., Factors affecting soil reaction. I. The soil-water ratio. Jour. Phys. Chem. 27:117–140. 1923.

[4] HARTER, L. L., and WEIMER, J. L., Amylase in the spores of *Rhizopus tritici* and *R. nigricans*. Amer. Jour. Bot. 10:89–92. 1923.

[5] WEIMER, J. L., and HARTER, L. L., Pectinase in the spores of *Rhizopus*. Amer. Jour. Bot. 10:167–169. 1923.

[6] WEIMER, J. L., and HARTER, L. L., Influence of temperature on the pectinase production of different species of *Rhizopus*. Amer. Jour. Bot. 10:127–132. 1923.

Monograph of Peronospora.—The fourth part of the fifth volume of the contributions to the cryptogamic flora of Switzerland is devoted to an elaborate study of the genus *Peronospora* by GÄUMANN.[8] The investigation extended over a period of five years and was completed in 1918, since which time it has been awaiting publication. To better institute comparisons, the species for the most part are brought together under the host families. Beside the usual technical description, synonymy, distribution, etc., almost every species is illustrated with very exact drawings of conidiospores, conidiophores, and biologic curves based on the length and the breadth of the spores. The dimensions of the spores were secured by taking the average of 500 measurements made with a Leitz graded micrometer. A large number of new species are described. Although primarily undertaken as a study of the Swiss flora, it was extended to include all known species in order to secure a broader and more accurate viewpoint. The general study is preceded by a systematic key to the Swiss species, numbering 142. The character of the oospore wall throws the species into two sub-families, and each of these into two groups. In the separation of species much stress is laid upon the size of the conidiospores. The minutiae and comprehensiveness of the study may be inferred from an introductory review of the species concept, especially as applied to the genus *Peronospora*, covering twenty-six pages, in which are inserted much experimental data. The work forms a notable contribution to cryptogamic literature.—J. C. ARTHUR.

Resin formation.—The formation of resin in conifer needles has been investigated by HANNIG,[10] who apparently overthrows TSCHIRSCH's theory of resin secretion which claimed the presence of a special resin-secreting membrane on the outside of the resin-forming cell. HANNIG is unable to find such membranes, and explains how improper methods of preparation may have produced TSCHIRSCH's "Schleimbeleg," or "resinogene Schicht." The protoplasm of the epithelial cells surrounding the resin ducts is believed by HANNIG to be the source of resin formation. The resin droplets are first seen on the surface of the protoplasm, just inside the walls of the cells, only on the sides bordering on the canal. Since these droplets lie between the cell wall and protoplasm, HANNIG thinks that turgor pressure may be important in forcing the resin through the cell wall into the canal. By microchemical tests it is shown that the droplets formed by the cells and the resin in the canals are chemically alike. An excellent review of the microchemistry of resins is given. Larger droplets are found in the secreting cells of young needles than of old ones, according to HANNIG. The new HAAS and HILL text (Vol. I) states that the greatest flow of resin occurs in the spring, when, of course, the needles are young.—J. B. RHINE.

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[7] HARTER, L. L., and WEIMER, J. L., The relation of the enzyme pectinase to infection of sweet potatoes by *Rhizopus*. Amer. Jour. Bot. **10**:245–258. 1923.

[8] GÄUMANN, ERNST, Beiträge zu einer Monographie der Gattung *Peronospora* Corda. Beiträge zur Kryptogamenflora der Schweiz 5^4:1–360. 1923.

[9] HANNIG, E., Untersuchungen über die Harzbildungen in Koniferen Nadeln. Zeitschr. Bot. **14**:385–421. 2192.

Bibliography of colloids.—An extensive bibliography of literature dealing with the colloidal state has been prepared for the National Research Council by HOLMES,[10] and published in mimeograph form. While the list is preliminary to a more complete bibliography, about 1900 titles are listed, dealing with every phase of the colloidal state. Among the classified groups are many of biological interest, such as adsorption, biocolloids, clays and soils, dialysis, emulsions, gels, gums, indicators, protein swelling, surface tension, ultra microscope, viscosity, etc. Investigators in this field will welcome the opportunity to secure accurately prepared and reasonably complete lists of citations on these topics. There is hardly a single field of science which does not at some point deal with colloidal behavior, and the National Research Council is rendering a notable service to science in having this bibliography prepared. The conservation of the time of the individual investigator is very important, and these aids to investigation materially increase the productivity in research.—C. A. SHULL.

Electrical stimulation.—A résumé of some experiments on electrical stimulation of plants is given by KOKETSU,[11] in which protoplasmic streaming is brought to a standstill, or the size of cells diminished. The responses were studied from various angles, such as rate of response, intensity effects, value of threshold stimulus, duration of required stimulus, etc. Changes in sensitivity, polar effects, galvano-tropisms, taxies, and tetanus effects were demonstrated. The author seems not to have been aware of the more extensive work of BOSE along these lines.—J. B. RHINE.

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[10] HOLMES, H. N., A bibliography of colloid chemistry. Nat. Res. Council, Washington, D.C. $1.00.

[11] KOKETSU, R., Über die Wirkung der elektrischen Reizung an den Pflanzlichen Gebilden. Bot. Mag. 36:129–132. 1922.

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ABSORPTION OF NUTRIENTS FROM SUBSOIL IN RELATION TO CROP YIELD

JOHN W. CRIST AND J. E. WEAVER

(WITH NINE FIGURES)

Investigations of the development of root systems of native and crop plants have been pursued for a number of years at the University of Nebraska. The gradual intensification of this work has led to a type of experimentation designed to discover the nature of the activities of roots at great depths in relation to absorption of water and nutrients. In the present investigation the effects of absorption of nutrients from deep levels upon quantity and quality of yield were determined. Studies on the amounts of water and nitrates removed by crops from various soil levels were begun in 1919, but little attention was then given to yield. Since the results of these earlier experiments have a direct bearing upon the present problem, however, the methods employed being essentially the same, the more important results will be briefly summarized (cf. WEAVER, JEAN, and CRIST **20**).

Containers 1.5–3 feet in diameter and 2.5–5 feet deep were employed. They were placed in trenches, which were then refilled with soil, and crops planted around the containers in such a manner that the experimental crops in the containers were grown under field conditions. The containers were filled with well mixed soil of known water content and physical and chemical composition, to which, at certain levels, $NaNO_3$ had been added at the rate of

400 parts per million. The containers were filled in such a manner that the well compacted soil at any level occupied the same relative position as regards depth that it had occupied before removal from the field. The fertilized layers, and in some cases every 6-inch layer, were separated from the rest of the soil by wax seals which prevented the movement of water or solutes, but through which the roots readily penetrated. To prevent water intake, each container was furnished with an appropriate wooden roof. Crops of Manchuria barley, early Ohio potatoes, and maize were grown. In order to study the activities of the roots at various stages in their development, enough containers were used so that some could be examined at each of the several periods of growth. In fig. 1, containers 1 and 2 illustrate diagrammatically the development of the roots of barley when 55 days old and in the 6 or 7 leaf stage. In containers 3, 4, and 5 the crop was in blossom (74 days old), while in 6 and 7 it had reached maturity. The horizontal lines indicate the positions of the wax seals, and the double vertical lines the positions of the 6-inch levels to which the nutrient was added. The numbers at the bottoms of the containers give the nitrates in parts per million, based on the dry weight of the soil, absorbed from the fertilized layers. The gains by nitrification at the several levels were determined from the control container (no. 8), without a crop.

The amount of water absorbed by barley from the deeper soil (to 3.5 feet) was in direct relation to the growth of the root system into these deeper layers. The total amounts absorbed to depths of 2.5 feet in general were practically the same from the several 6-inch levels. Corn is an extravagant user of water, absorbing large quantities from the third and fourth foot of soil, and smaller amounts from the fifth foot. Potatoes absorbed water to depths of 2.5 feet. Potatoes used the nitrates in smaller amounts than barley. When beginning to blossom (74 days old) they had removed 143 and 70 parts per million of nitrates from the 1–1.5 and 1.5–2 foot layers respectively, and when beginning to ripen (100 days old) they had removed 228 parts per million at a depth of 1.5–2 feet, and 76 to 165 parts per million at the 2–2.5 foot level. Corn removed 203, 140, and 118 parts per million at depths of 3, 4, and 5 feet respectively.

From these experiments we may conclude that (1) the roots of crop plants are active in the absorption of both water and nitrate salts, even at the maximum depth of their penetration; (2) both water and nitrates are taken up from these lower portions of the soil in considerable quantities, although to a less extent than from the more superficial portions; (3) the plants receive this supply of water and nitrogen from the deeper soil layers during the later and perhaps more critical stages of their development; (4) the roots branch more profusely and are more abundant where they come in contact with the fertilizer; and (5) there is urgent need for a series of experiments to ascertain the significance of absorption at great depths in relation to quantity and quality of crop yield.

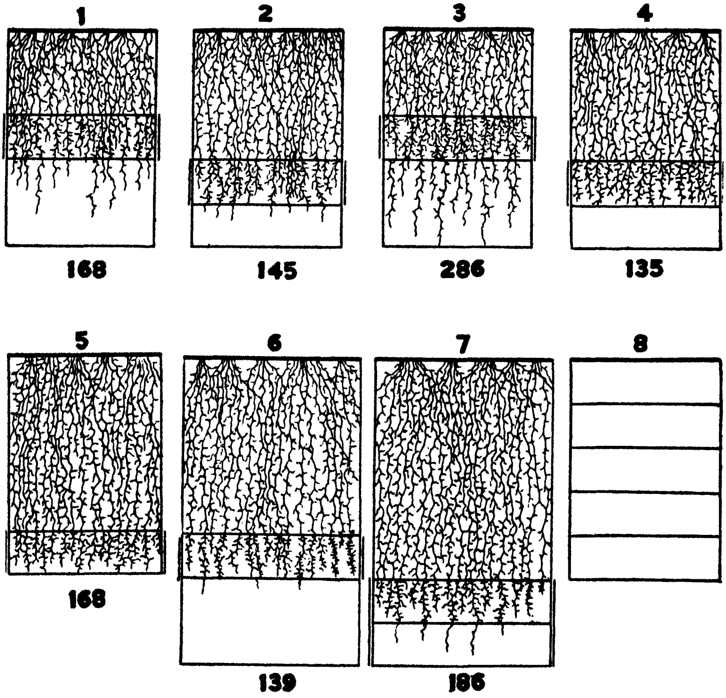

Fig. 1.—Diagrammatic representation of root development of barley at three different stages in growth; NaNO$_3$ occurred at several levels delimited by double vertical lines and horizontal lines, latter represent wax seals.

An extensive literature elucidating the problem of fertilizers and their effects upon yield of the various economic crops has been produced. Practically none of it refers to the present type of investigation, because in all experimental tests and agricultural practices the application of fertilizer has been restricted to the surface or merely to the first four to eight inches of the soil. Among soil scientists it has long been the custom to take samples to depths as great as 4–6 feet and occasionally even deeper. While slight attention has usually been given to the composition of the deeper samples, yet it has meant a degree of recognition of the importance of the nature of the deeper soil and soil solution. Serious consideration of the lower subsoil has been taken only when it was of rock or contained a hardpan, or, in some other way than through a deficiency of nutrient substances became detrimental to crop production (19).

Experimental methods

Large, water tight oak barrels 22 inches in minimum diameter and 30 inches deep were used as containers. Sufficient soil to fill them was obtained from the first 2.5 feet of the soil in the field in which the experimental work was done. The soil was low in both nitrate nitrogen and active phosphoric acid (table I). While crops on eastern Nebraska soils respond readily and consistently to nitrate fertilizers, experiments conducted by the department of agronomy of the University of Nebraska (unpublished data) have shown that phosphorus fertilizers have failed to stimulate production.

TABLE I

NO_3 AND P_2O_5 CONTENT OF SOILS

Soil	Depth (ft.)	Nitrate (NO_3) (p.p.m.)	P_2O_5 (per cent)
Surface..........	0–1	99.5	0.029
Subsoil..........	1–2.5	11.1	0.012

On March 27, 1922, two trenches were dug in the field where the crops were to be grown, and the containers, 18 in number, placed in them in rows with the tops about 4 inches above the general soil level. The soil had previously been brought to an approximately uniform water content of about 27 per cent. As the several containers were filled, samples were taken at each level for moisture determinations, and also, where necessary, for the determination of nitrates. The fertilized layers were separated from the soil, both above and below, by means of wax seals, which consisted of 85 per cent paraffin and 15 per cent petrolatum. The seal was applied hot, so that it penetrated a little into the soil, and when it cooled clung tenaciously to the soil particles. It varied from 2 to 3 mm. in thickness. As shown in previous investigations, the seal has no effect upon root development (20). The nutrient salts used were chemically pure monocalcium phosphate and sodium nitrate. The phosphate was applied at the rate of 1 gm. to 30 lb. of soil, which is approximately equivalent to 500 pounds of acid phosphate per acre. The nitrate was added at the rate of 292 parts per million of NO_3 on the basis of the dry weight of the soil.

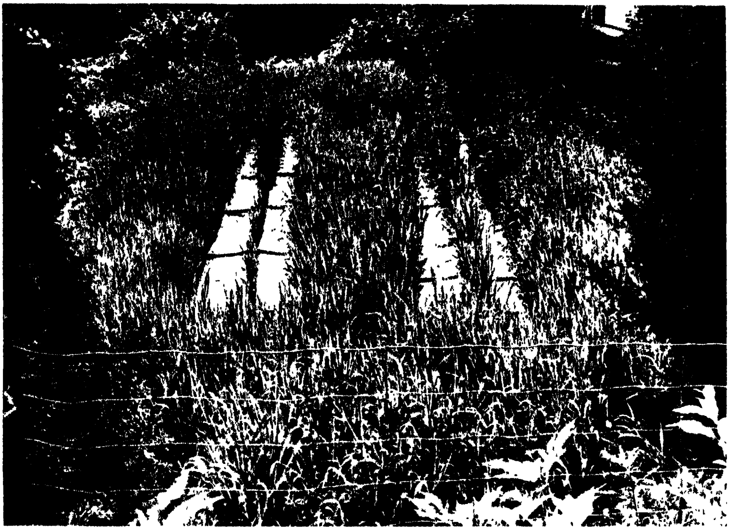

FIG. 2.—General view of experimental plat on May 29.

When the containers had been filled to within 2 inches of the top, two thin wooden strips, about 1.5 inches in width and as long as the diameters of the containers, were placed edgewise and partially sunk into the soil so as to prevent the wax seal from covering the area of soil (about 1.5 inches in width) in which the crops were to be planted. Finally, the wax seal was covered with 2 inches of sand. Next a light green wooden roof, with sufficient slope to cause the water to run off, but with an opening 1.5 inches wide and as long as the diameter of the container, was fastened in place. A small amount of soil was placed in the openings thus left, and after the crop was planted about an inch of dry soil added to check evaporation (fig. 2). Seeds of Manchuria barley, selected for uniformity in size and previously treated with formalin solution to prevent smut infection, were sown on April 7, 100 seeds per container.

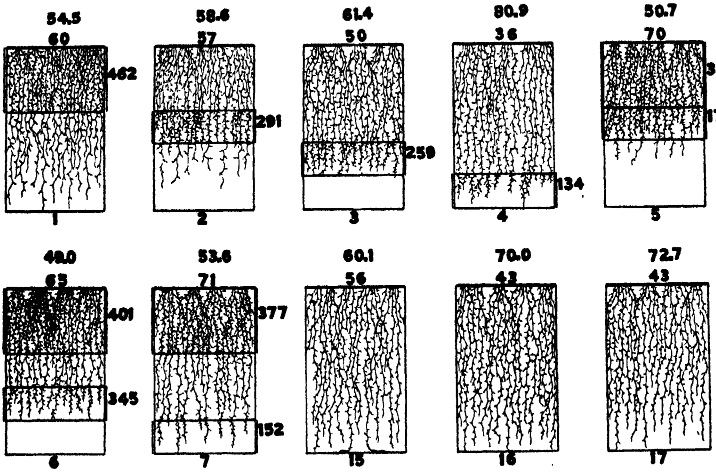

FIG. 3.—Diagrammatic representation of root development of barley fertilized with $NaNO_3$; seals and position of fertilizer indicated as in fig. 1; numbers immediately above containers represent number of plants, those higher, average length of stalks in centimeters.

The position of the fertilized layers in the several containers in the nitrogen series, together with the unfertilized control crops, is shown in fig. 3, which also illustrates the relative root development at the time of harvest, June 26. The positions of the wax seals are indicated by horizontal lines and that of the fertilizer by double vertical lines. A control container without a crop or fertilizer, but sealed at every 6-inch level, was used as a check to determine the extent of nitrification and denitrification. Root development, etc., at the time of harvest on June 24, is shown for the phosphorus series in fig. 4.

On May 2, when germination was completed and most of the seedlings had reached the second leaf stage, they were thinned to 35 per container. Eleven days later, just as tillering was beginning, enough of the smaller plants were removed to leave only 30 plants in each container, a number not in excess of that growing in a similar soil area under field conditions. The growth of the crops in the containers, as in previous experiments, paralleled that of the crop surrounding them, except for such differences as were caused by the fertilizer, which could readily be observed upon comparison with the controls. On May 22, and again on June 17, when very hot dry weather prevailed, the experimental crops were watered, the same amount of water (1 liter on the first date and 3 liters on the

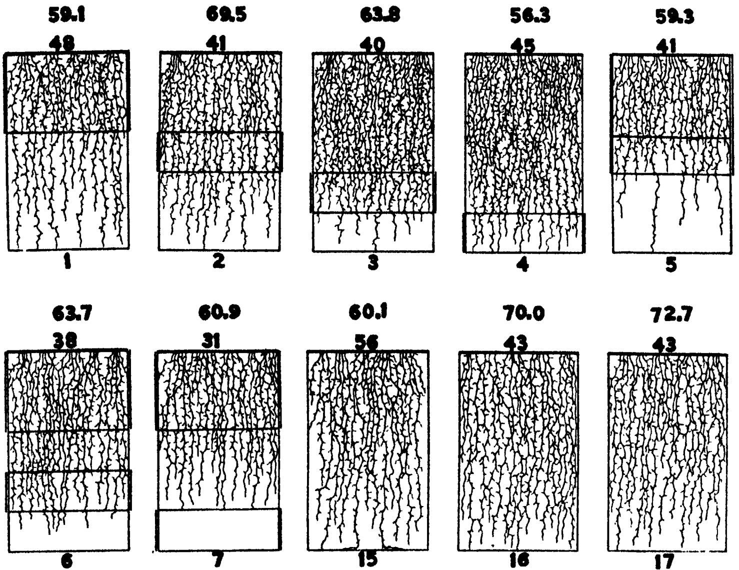

Fig. 4.—Diagrammatic representation of root development of barley fertilized with $CaH_4(PO_4)_2$; seals, position of fertilizer, number of plants, and length of stalks indicated as in fig. 3.

second) being poured slowly into each container through the narrow openings through which the plants were growing. By June 1 the crop was heading. June 10–22 was a period of dry and unusually hot weather, which resulted in the general harvest of eastern Nebraska being at least 10 days earlier than usual. The experimental crops ripened very rapidly, and were harvested on June 24 and 26 respectively, the phosphorus series maturing first.

In harvesting the crop, the plants were cut off at the surface of the soil. Those from each container were wrapped separately in muslin cloths and taken to the laboratory, where they were spread out on tables in a well ventilated, well lighted room and allowed to dry. When thoroughly dry, the various measurements were taken, weights determined, and analyses made. In taking down the containers, they were tilted on their sides in the trench, the hoops chiseled off, the top staves removed, and the exposed core of the soil taken out in 6-inch sections in examining root development and obtaining samples for analyses. All soil samples for chemical analyses were taken in large composite form. Those to be analyzed for nitrates were treated with small amounts of toluol and sealed at once in mason jars. In the nitrate determinations the method of WHITING (21) was employed. Phosphorus determinations were made by digestion in fifth normal nitric acid for 5 hours at 40° C. This gave the quantity of the so-called active phosphoric acid (FRAPS 6). The usual modified Gunning method was used in the analyses of grain and straw for nitrogen. Determinations of phosphorus in the grain and straw were made by digesting the samples with concentrated nitric acid in the presence of MgO, followed by precipitation from the extract with ammonium molybdate and the other steps in the gravimetric process.

Experimental results

NITRATE SERIES

The growth of root and shoot and the amounts of water and nitrates removed by the crop in the nitrate series, together with the water loss from the controls, are shown in table II. These data show the remarkable activity of roots in the absorption of both water and nitrates in the lower soil levels. Notwithstanding the

TABLE II

LOSS OF WATER AND NITRATES APRIL 7 TO JUNE 26

CONTAINER AND CROP DEVELOPMENT	DEPTH IN FEET	WATER CONTENT			NITRATE (NO_3) PARTS PER MILLION					
		April 7 (per cent)	June 26 (per cent)	Loss (per cent)	Original content April 7	Impregnation April 7	Gain by nitrification	Total	June 26	Loss
No. 1: 60 stalks, average length 54.5 cm., 42 heads; roots well developed in first foot, abundant to 1.5 feet, and fairly abundant to 2.5 feet	0–1	22.3	11.2	11.1	99.5	356.8	70.7	527	64.4	462.6
	1–1.5	22.4	16.9	5.5	......	......	......	......	......	......
	1.5–2	23.8	17.1	6.7	......	......	......	......	......	......
	2–2.5	23.3	18.3	5.0	......	......	......	......	......	......
No. 2: 57 stalks, average length 58.6 cm., 43 heads; roots well developed in first foot, very abundant at 1–1.5 feet, but sparse to 2 feet, a few extended to 2.1 feet	0–1	24.8	11.1	13.7	......	......	......	......	......	......
	1–1.5	22.6	16.8	5.8	11.1	362.9	24.9	398.9	107.5	291.4
	1.5–2	22.8	18.0	4.8	......	......	......	......	......	......
	2–2.5	25.2	25.0	0.2	......	......	......	......	......	......
No. 3: 50 stalks, average length 61.4 cm., 40 heads; normal development of roots to 1.5 feet; then very abundant and evenly distributed to 2 feet, below which practically none occurred	0–1	22.5	11.8	10.7	......	......	......	......	......	......
	1–1.5	25.3	18.0	7.3	......	......	......	......	......	......
	1.5–2	25.2	17.8	7.4	11.1	365.3	25.8	402.2	142.4	259.8
	2–2.5	23.2	23.2	0.0	......	......	......	......	......	......
No. 4: 36 stalks, average length 80.9 cm., 36 heads; roots abundant and evenly distributed to 2 feet, below which abundant but not uniformly distributed	0–1	23.4	12.2	11.2	......	......	......	......	......	......
	1–1.5	25.4	16.4	9.0	......	......	......	......	......	......
	1.5–2	23.7	17.9	5.8	......	......	......	......	......	......
	2–2.5	25.7	20.0	5.7	11.1	369.6	24.5	405.2	270.9	134.3
No. 5: 70 stalks, average length 50.7 cm., 47 heads; roots very abundant, profusely branched, uniformly distributed to 1.5 feet; few below this level and none beyond 1.8 feet	0–1	23.9	11.9	12.0	99.5	361.5	70.7	531.7	179.9	351.8
	1–1.5	24.3	17.4	6.9	11.1	361.8	24.9	397.8	222.3	175.5
	1.5–2	26.2	22.3	3.9	......	......	......	......	......	......
	2–2.5	23.4	22.9	0.5	......	......	......	......	......	......
No. 6: 65 stalks, average length 49 cm., 41 heads; roots well developed to 1.5 feet, fairly abundant and exceptionally well branched at 1.5–2 feet, none deeper	0–1	23.1	11.3	11.8	99.5	364.7	70.7	534.9	133.4	401.5
	1–1.5	25.0	16.6	8.4	......	......	......	......	......	......
	1.5–2	25.4	22.2	3.2	11.1	378.4	25.8	415.3	69.7	345.6
	2–2.5	22.9	22.5	0.4	......	......	......	......	......	......
No. 7: 71 stalks, average length 53.6 cm., 49 heads; roots very abundant, uniformly distributed to 1.5 feet, fairly abundant to 2 feet, not exceptionally abundant below 2 feet but exceedingly well branched	0–1	23.7	11.6	12.1	99.5	363.5	70.7	533.7	156.5	377.2
	1–1.5	24.6	16.5	8.1	......	......	......	......	......	......
	1.5–2	25.0	18.3	6.7	......	......	......	......	......	......
	2–2.5	29.8	24.9	4.9	11.1	378.7	24.5	414.3	261.9	152.4
Container without crop to determine nitrification and denitrification	0–1	25.0	23.9	1.1	26.6	......	70.7	97.3	97.3	......
	1–1.5	25.3	25.2	0.1	8.8	......	24.9	33.7	33.7	......
	1.5–2	25.7	24.4	1.3	22.1	......	25.8	47.9	47.9	......
	2–2.5	25.4	26.0	+0.6	8.8	......	24.5	33.3	33.3	......

TABLE II—*Continued*

Container and crop development	Depth in feet	Water content			Nitrate (NO_3) parts per million					
		April 7 (per cent)	June 26 (per cent)	Loss (per cent)	Original content April 7	Impregnation April 7	Gain by nitrification	Total	June 26	Loss
No. 15: 56 stalks, average length 60.1 cm., 41 heads; normal development of roots to 2 feet; fewer roots deeper but some reached 2.5 feet and ran along bottom of container	0–1	24.2	11.7	12.5	Unfertilized					
	1–1.5	24.9	16.8	8.1						
	1.5–2	24.8	17.8	7.0						
	2–2.5	27.4	21.7	5.7						
No. 16: 43 stalks, average length 70.0 cm., 38 heads; roots very abundant in first 2 feet; fewer below, but extended to 2.5 feet	0–1	24.3	12.0	12.3	Unfertilized					
	1–1.5	23.9	16.9	7.0						
	1.5–2	23.9	16.4	7.5						
	2–2.5	27.6	21.0	6.6						
No. 17: 43 stalks, average length 72.7 cm., 38 heads; roots very abundant to 2 feet, and fairly abundant to bottom of container	0–1	24.6	11.2	13.4	Unfertilized					
	1–1.5	22.6	14.7	7.9						
	1.5–2	23.8	17.8	6.0						
	2–2.5	27.3	23.2	4.1						

rapid ripening of the crop, due to abnormal weather conditions, water was absorbed in considerable quantities (3–13 per cent of dry weight of soil) at all depths to which the roots penetrated. The large amounts of nitrates removed at the several levels are indicated by numbers representing parts per million opposite these levels in fig. 3. When the fertilizer was placed both in the surface foot and at 1–1.5, 1.5–2, and 2–2.5 feet respectively, the amounts removed were, in order, 14.0, 61.5, and 14.5 per cent greater than when the fertilizer was placed in the surface foot alone.

The number of stalks developing from the 30 original plants in each container decreased progressively with the depth of the fertilizer from 60 to 36 (fig. 3). Except when the nitrates were below 2 feet, the number of stalks was greater than that of the average of the controls. In the doubly fertilized series the number varied from 65 to 71, which was 38–51 per cent greater than the average of the controls, and 8–18 per cent greater than when the fertilizer was placed in the surface foot only.

The average length of stalk increased progressively as the fertilizer was more deeply placed, ranging from 54.5 to 80.9 cm. (fig. 3). Except when the nitrates were below 2 feet in depth, the stalks averaged shorter than those of the controls. In the doubly fertilized series the stalks were not only shorter than those of any of the controls, but also shorter than those of any of the singly fertilized series.

The number of heads was greater in every container (except no. 4) than the average of the controls. Where double fertilization obtained, the number exceeded that of the controls by 5–26 per cent. This statement may be misleading, however, unless the differences in the total number of stalks are taken into account. Column 7 of table III gives the percentage of stalks bearing heads, and shows that in every container (except no. 4, where the fertilizer was at 2–2.5 feet and was reached at a later period) a smaller percentage of stalks bore heads, and that the smallest percentages are associated with the doubly fertilized soils. The heads in the doubly fertilized series were longest and exceeded the average of controls by 6–12 per cent.

TABLE III

NUMBER AND LENGTH OF STALKS AND HEADS IN NITRATE SERIES

Container	Depth of fertilizer (feet)	Number of stalks	Average length of stalks (cm.)	Number of heads	Average length of heads (cm.)	Stalks bearing heads (per cent)
1......	0.1	60	54.5	42	6.9	70
2......	1–1.5	57	58.6	43	6.7	75
3......	1.5–2	50	61.4	40	6.1	80
4......	2–2.5	36	80.9	36	6.4	100
5......	0–1 and 1–1.5	70	50.7	47	6.9	67
6......	0–1 and 1.5–2	65	49.0	41	7.3	63
7......	0–1 and 2–2.5	71	53.6	49	7.2	69
15......	....	56	60.1	41	6.7	73
16......	....	43	70.0	38	6.4	90
17......	....	43	72.7	38	6.4	90

The average total dry weight of the controls (86.9 gm.) was exceeded by 12.2 per cent when the fertilizer was added to the surface foot only (table IV), but when the fertilizer was placed at lower levels, a decrease of 5.9, 15.5, and 4.9 per cent was determined in containers 2, 3, and 4 respectively. Adding nitrates to other levels in addition to the first foot resulted in an increase in dry weight. This gain amounted to 11.5, 11.3, and 26.9 per cent over the average of the controls in containers 5, 6, and 7 respectively.

The dry weight of the grain was decreased in every case where nitrate was applied either singly or doubly, except in container 7. This decrease was no greater in the doubly fertilized than in the singly fertilized series, nor is there any definite relation between the depth of the fertilizer and the depression in yield of grain.

The dry weight of straw was increased in container 1 of the singly fertilized series 24.9 per cent over the average of the controls, but where the nitrate was placed deeper than the first foot, a decrease occurred. The dry weight was equal to that of each of two of the controls, however, where the salt was at 1–1.5 and at 2–2.5 feet. The increases in the doubly fertilized soils were very marked in every instance, being 27.9, 27.9, and 40.8 per cent in containers 5, 6, and 7 respectively. Furthermore, the increase in the doubly fertilized series over that of the crop where the soil was fertilized only in the surface foot was 2.4–12.7 per cent.

TABLE IV

DRY WEIGHT AND NITROGEN CONTENT OF GRAIN AND STRAW

Container	Depth of fertilizer (feet)	Dry weight of straw (gm.)	Dry weight of grain (gm.)	Total dry weight of tops (gm.)	Ratio of grain to straw	Nitrogen content of grain (per cent)	Nitrogen content of straw (per cent)
1........	0.1	67.1	30.4	97.5	0.45	3.19	2.01
2........	1–1.5	52.9	28.9	81.8	0.55	3.30	1.42
3........	1.5–2	46.3	27.1	73.4	0.59	2.52	0.99
4........	2–2.5	51.1	31.5	82.6	0.62	2.53	0.82
5........	0–1 and 1–1.5	68.7	26.2	96.9	0.41	3.39	1.92
6........	0–1 and 1.5–2	68.7	28.0	96.7	0.41	3.41	1.82
7........	0–1 and 2–2.5	75.6	34.7	110.3	0.46	3.38	2.02
15........	None	58.7	33.4	92.1	0.57	2.79	0.86
16........	None	51.2	31.6	82.8	0.62	2.47	0.66
17........	None	51.3	34.7	86.0	0.68	2.33	0.55

The application of nitrogen fertilizer caused reduction of grain in proportion to straw. From the data in column 6, table IV, it may be seen that the ratio of grain to straw is lower than the average of the controls in any container in which the crop was fertilized. The ratio increased as the amount of nitrate absorbed decreased. Thus it rose from 0.45, when the nitrates were in the surface foot, progressively to 0.62, when the nitrates were at a depth of 2.5 feet. It was less in the doubly fertilized series (except in container 7) than when the nitrate was added to the surface foot only.

A comparison of the data on the nitrogen content of grain and straw given in table IV shows that (1) the average nitrogen content of the grain of the controls (2.53 per cent) was exceeded by that of the crop from the singly fertilized series by 26.1 and 30.4 per cent respectively, when the fertilizer was at depths of 0–1 and 1–1.5 feet; (2) in the doubly fertilized soils the greater nitrogen content of the grain over the average of the controls was 34, 34.8, and 33.6 per cent respectively, as the salt was progressively deeper. Moreover, only one of the controls exceeded in nitrogen content of grain any individual of either of the two fertilized series. The grain from container 15 analyzed higher than that from containers 3 and 4. The average nitrogen content of the straw of the controls (0.69 per cent) was exceeded in every case by that of the fertilized plants. Moreover, any one of the controls had a much lower nitrogen content of straw than any crop in the fertilized series, except where the nitrate was below two feet. The increases, in sequence from containers 1 to 4, were 191.3, 105.8, 43.5, and 18.8 per cent, and for the doubly fertilized series (5 to 7) 178.2, 163.7, and 192.7 per cent.

PHOSPHORUS SERIES

The experiment with phosphorus fertilizer was identical with that of the nitrogen series as regards number of containers, position of seals, impregnated soil layers, etc., and the same control crops were used for comparison. The growth of the tops and roots, together with the amounts of water removed at the several levels, is shown in table V. A comparison of this table with fig. 4 shows the root activities at all depths, water being absorbed in quantities similar to those removed by plants of the nitrate series.

Data on the number and length of stalks and heads in the several containers are given in table VI. The number of stalks and the length of heads were less than the average of the controls in every case where fertilizer was used, except in the first container. Moreover, with the exception of container 2, the stalks were shorter and the heads fewer, but the percentage of stalks bearing heads was nearly equal to or greater than that of the average of the controls. In the containers where the soil was fertilized at two levels the stalks averaged fewer, and the heads were fewer and shorter than when phosphorus was placed in a single layer. With one exception, however, the percentage of stalks bearing heads was greater in the doubly fertilized series.

TABLE V

LOSS OF WATER FROM PHOSPHORUS SERIES AND CONTROLS APRIL 7 TO JUNE 24

CONTAINER AND CROP DEVELOPMENT	DEPTH IN FEET	WATER CONTENT			POSITION OF FERTILIZER (feet)
		April 7 (per cent)	June 24 (per cent)	Loss (per cent)	
No. 1: 48 stalks, average length 59.1 cm., 38 heads; roots as abundant as in other unfertilized first foot of soil; below 1 foot less abundant than usual but extending to 2.5 feet	0–1	21.9	10.9	11.0	0–1
	1–1.5	23.1	17.8	5.3	
	1.5–2	24.8	19.9	4.9	
	2–2.5	28.1	25.5	2.6	
No. 2: 41 stalks, average length 69.5 cm., 39 heads; roots fairly abundant in first 1.5 feet, below which much fewer but some extended to 2.5 feet	0–1	23.9	11.9	12.0	1–1.5
	1–1.5	25.3	20.4	4.9	
	1.5–2	23.5	21.1	2.4	
	2–2.5	25.0	23.2	1.8	
No. 3: 40 stalks, average length 63.8 cm., 33 heads; roots very abundant, well distributed to 1.5 feet; fairly abundant to 2 feet, but sparse 2–2.5 feet	0–1	27.0	11.6	15.4	1.5–2
	1–1.5	24.8	17.3	7.5	
	1.5–2	22.4	19.6	2.8	
	2–2.5	25.8	24.2	1.6	
No. 4: 45 stalks, average length 56.3 cm., 37 heads; roots very abundant in first foot and more abundant to 2 feet than in any container of series; fairly abundant at 2–2.5 feet	0–1	24.2	12.2	12.0	2–2.5
	1–1.5	23.0	13.6	9.4	
	1.5–2	23.3	18.0	5.3	
	2–2.5	28.3	23.6	4.7	
No. 5: 41 stalks, average length 59.3 cm., 35 heads; roots very abundant, evenly distributed to 1.5 feet; very few found deeper, single root penetrated to 2.5 feet	0–1	25.1	12.9	12.2	0–1 and 1–1.5
	1–1.5	24.1	20.0	4.1	
	1.5–2	23.3	21.1	2.2	
	2–2.5	25.2	24.1	1.1	
No. 6: 38 stalks, average length 63.7 cm., 33 heads; roots very abundant, uniformly distributed to 2 feet, scarce in 2–2.5 foot layer except in center where they penetrated to 2.4 feet	0–1	26.5	11.9	14.6	0–1 and 1.5–2
	1–1.5	29.0	19.0	10.0	
	1.5–2	24.1	16.0	8.1	
	2–2.5	25.6	24.3	1.3	
No. 7: 31 stalks, average length 60.9 cm., 28 heads; roots abundant, rather evenly distributed to 1.5 feet, slightly fewer to 2 feet, below which none found	0–1	24.4	11.8	12.6	0–1 and 2–2.5
	1–1.5	26.2	19.1	7.1	
	1.5–2	25.2	20.9	4.3	
	2–2.5	23.6	23.0	0.6	
No. 15: 56 stalks, average length 60.1 cm., 41 heads; normal development of roots to 2 feet; fewer deeper but some reached 2.5 feet and ran along bottom of container	0–1	24.2	11.7	12.5	None
	1–1.5	24.9	16.8	8.1	
	1.5–2	24.8	17.8	7.0	
	2–2.5	27.4	21.7	5.7	
No. 16: 43 stalks, average length 70.0 cm., 38 heads; roots very abundant in first 2 feet of soil; fewer below but extending to 2.5 feet	0–1	24.3	12.0	12.3	None
	1–1.5	23.9	16.9	7.0	
	1.5–2	23.9	16.4	7.5	
	2–2.5	27.6	21.0	6.6	
No. 17: 43 stalks, average length 72.7 cm., 38 heads; roots very abundant to 2 feet and fairly abundant to bottom of container	0–1	24.6	11.2	13.4	None
	1–1.5	22.6	14.7	7.9	
	1.5–2	23.8	17.8	6.0	
	2–2.5	27.3	23.2	4.1	

Table VII shows that the average total dry weight of the controls (87.0 gm.) exceeded that of any of the crops where the soil was fertilized at any depth with phosphorus. In the singly fertilized soils, in the order of the increasing depth of the fertilizer, the decreases were 11.3, 18.1, 24.7, and 23.7 per cent respectively. Where doubly fertilized the depression in yield was much greater, being, in the above order, 43.0, 36.6, and 51.6 per cent. These great decreases in dry weight occurred both in grain and straw. The depression of grain yield was somewhat greater than that of the straw when only one layer of soil was fertilized, but when a deeper layer in addition to the surface foot was impregnated, the depression of the straw yield was greater.

TABLE VI

NUMBER AND LENGTH OF STALKS AND HEADS IN PHOSPHORUS SERIES

Container	Depth of fertilizer (feet)	Number of stalks	Average length of stalks (cm.)	Number of heads	Average length of heads (cm.)	Stalks bearing heads (per cent)
1......	0–1	48	59.1	38	6.5	79.1
2......	1–1.5	41	69.5	39	5.5	95.1
3......	1.5–2	40	63.8	33	6.4	82.5
4......	2–2.5	45	56.3	37	6.0	82.2
5......	0–1 and 1–1.5	41	59.3	35	4.6	85.5
6......	0–1 and 1.5–2	38	63.7	33	5.1	86.8
7......	0–1 and 2–2.5	31	60.9	28	5.2	90.3
15......	Control	56	60.1	41	6.7	73.2
16......	Control	43	70.0	38	6.4	88.3
17......	Control	43	72.7	38	6.4	88.3

TABLE VII

DRY WEIGHT AND PHOSPHORUS CONTENT OF GRAIN AND STRAW

Container	Depth of fertilizer (feet)	Dry weight of straw (gm.)	Dry weight of grain (gm.)	Total dry weight of tops (gm.)	Ratio of grain to straw	Phosphorus content of grain (per cent)	Phosphorus content of straw (per cent)
1........	0.1	48.9	28.2	77.1	0.58	1.337	0.444
2........	1–1.5	43.9	27.3	71.2	0.62	1.245	0.476
3........	1.5–2	40.8	24.7	65.5	0.61	1.248	0.355
4........	2–2.5	40.5	25.9	66.4	0.64	1.176	0.418
5........	0–1 and 1–1.5	28.8	20.8	49.6	0.72	1.237	0.457
6........	0–1 and 1.5–2	33.9	21.2	55.1	0.63	1.237	0.706
7........	0–1 and 2–2.5	22.5	19.6	42.1	0.87	1.250	0.533
15........	None	58.7	33.4	92.1	0.57	1.210	0.287
16........	None	51.2	31.6	82.9	0.62	1.198	0.270
17........	None	51.3	34.7	86.1	0.68	1.179	0.347

The ratio of grain to straw averaged 0.62 for the controls. In the singly fertilized series variations from this ratio were negligible, but in the doubly fertilized soils the ratio was considerably increased.

Lack of consistent correlation of the ratios with the phosphorus content of either grain or straw, however, indicates that they probably have little significance. The average phosphorus content of grain of the controls (1.196 per cent) was less than that from any container where the soil was singly fertilized above 2 feet. The increases, in the sequence of increasing depth of fertilizer, were 11.8, 4.1, and 4.3 per cent. Where the phosphorus salt was below 2 feet a decrease of 1.7 per cent was determined. In the doubly fertilized soils the gains, in the above sequence, were 3.4, 3.4, and 4.5 per cent. Furthermore, with the single exception just noted, none of the three controls equaled in phosphorus content of grain that of any of the fertilized crops.

The increase of phosphorus content of straw over that of the controls, which averaged 0.301 per cent, was greater than that of the grain. Moreover, each control had a lower phosphorus content of straw than that of any crop regardless of the depth at which the fertilizer was present. In the singly fertilized series, in order of increasing depth of phosphorus salts, the percentages were 47.5, 58.1, 17.9, and 38.9 respectively. Using the same sequence in the doubly fertilized series, they were 51.8, 134.5, and 77.1 per cent.

Discussion

The value of the data obtained from these experiments is enhanced by the fact that the crop was grown under field conditions and in containers sufficiently large to permit normal root development. Except for the effects brought about by the fertilizers, the crop developed normally. This was determined both by comparison with the controls and the crop in the field surrounding the containers. The effects of the various treatments of fertilizer became evident in the nitrogen series early in the development of the crop. Not only were the plants in contact with the nitrates more numerous, but also more bushy. They had longer and broader leaves, slightly thicker stems, and were a deeper green than either the controls or the plants fertilized with phosphorus. All of these characters were accentuated in the containers where nitrates occurred in the deeper soils as well as in the surface foot. Plants in contact with the phosphorus showed a decided glaucous appearance.

These differences in the two series, when the crop was 53 days old, are shown in figs. 5 and 6. The lateral spread of the tops of the plants on May 27 averaged 10 inches for the controls, 7.5 inches for the phosphorus series, and 13 inches for those fertilized with nitrates. The average greatest diameter of the second leaf from the top at this time was 14.4 mm. for the controls, and 15 mm. and 18 mm. respectively for the phosphorus and nitrate series. Heading occurred among the phosphorus fertilized plants on June 1, slightly in advance of the controls, and 5 to 7 days earlier than in the nitrate series. The condition of the crop at the time of harvest (June 24), where the nitrate and phosphate respectively occurred at 1–1.5 feet in depth, as well as that of a control, is shown in figs. 7–9.

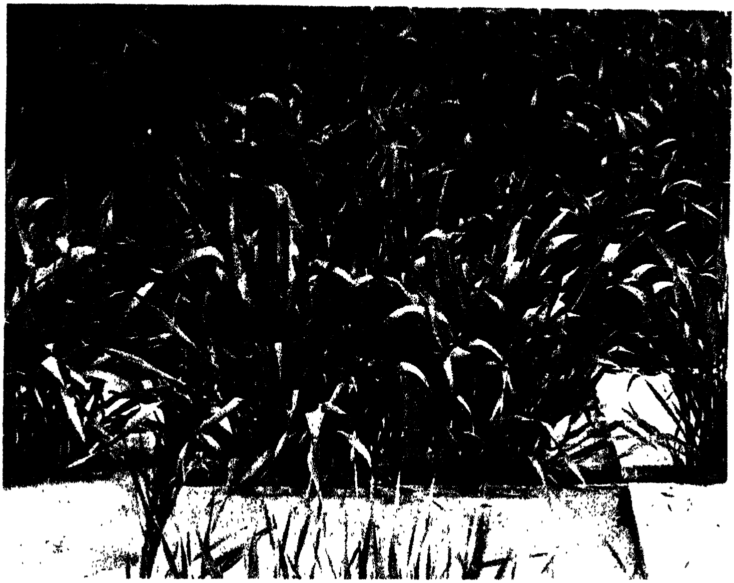

FIG. 5.—53-day old plants in container where soil was fertilized with nitrates at 0–1 and 1–1.5 feet.

The researches of NOBBE (16), THIEL (18), HÖVELER (11), FRANK (5), MÜLLER-THURGAU (15), VON SEELHORST (17), LIVINGSTON (14), BRENCHLEY and JACKSON (1), and many others are in agreement that the presence of fertilizer increases root development. A greater development of the barley roots in the soil layers impregnated with the nitrate was found, but little or no difference was observed in the phosphorus series.

It is of interest that the roots absorbed nitrates at all levels into which they penetrated, notwithstanding the fact that the salt at all times was abundant in the surface soil. In fact, they extracted almost as much from the surface foot in addition to the supply obtained from the deeper soil as when nitrate was supplied to the surface foot alone. This shows that the presence of salts in the subsoil profoundly affects the amount of nitrate absorbed, and this in turn materially affects the growth of the crop and the quantity and quality of the yield. The amount of phosphorus absorbed from the several levels was not determined, but judging from the variations in the phosphorus content of the plants, these statements seem to hold equally true for phosphate fertilizers.

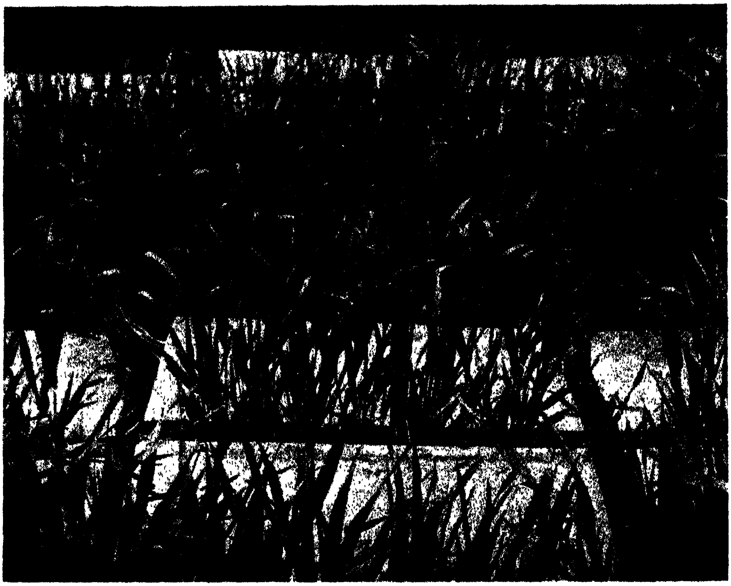

FIG. 6.—Soil fertilized with phosphorus at 0–1 foot

Nitrates placed below but in conjunction with fertilizer in the surface foot failed to increase the nitrogen content of the straw beyond that of the crop fertilized in the surface foot only. In every instance, however, it resulted in an increased nitrogen content of the grain. Conversely, under similar conditions when phosphorus fertilizer was employed, while the phosphorus content of the grain was not raised, that of the straw was increased in every case. Since phosphorus placed below as well as in the surface foot gave a better quality of straw, while under the same conditions

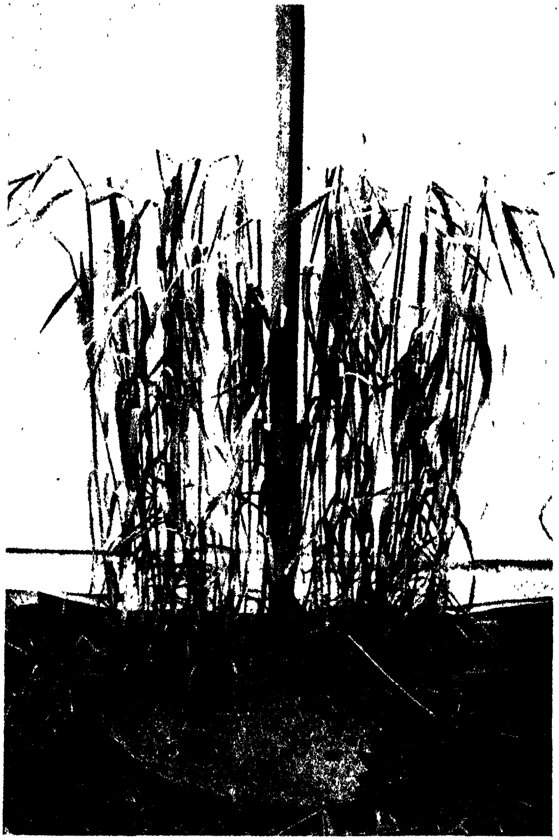

FIG. 7.—Mature crops: soil fertilized with nitrates

nitrogen caused a better quality of grain, it seems clear that when the limits of improvement in the quality of cereal crops through attention to the upper part of the soil have been reached, there are still possibilities of further progress through increased attention to the deeper strata.

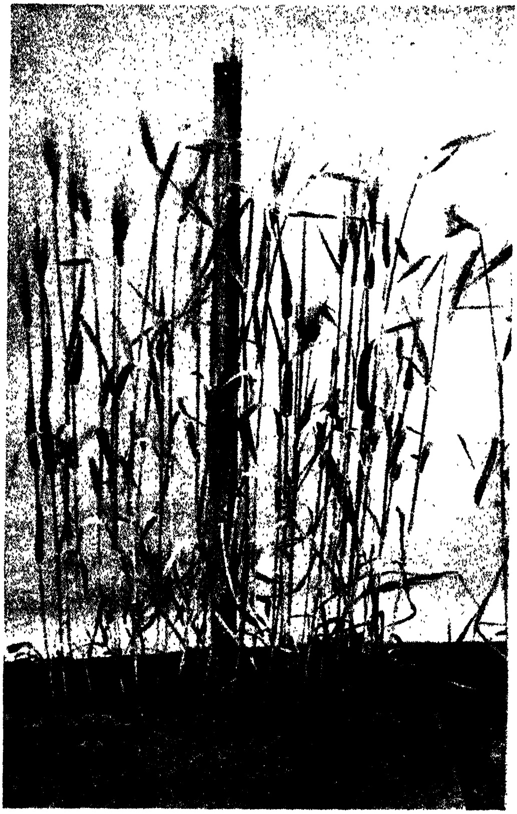

FIG. 8.—Mature crops: with phosphates at 1–1.5 foot depth respectively

That nitrogen promotes tillering is well known. These experiments show that the addition of more nitrogen below the surface foot makes the tillering habit even more pronounced. GERICKE (7), in accounting for the abundant tillering and culm production in cultures of wheat which received additional supplies of nitrogen late in the growing period, concluded that it was due to the greater extent of the root system. This resulted in the absorption of a larger amount of nutrients than was needed by the plants for normal development of the individual shoots. Our results are in agreement with this explanation. The number of stalks followed closely the amount of nitrate absorbed, and even more closely the nitrogen content of both grain and straw at maturity. Moreover, the amount of tillering correlated closely with the root area in contact with the nutrient, if we consider that the extent of the root systems

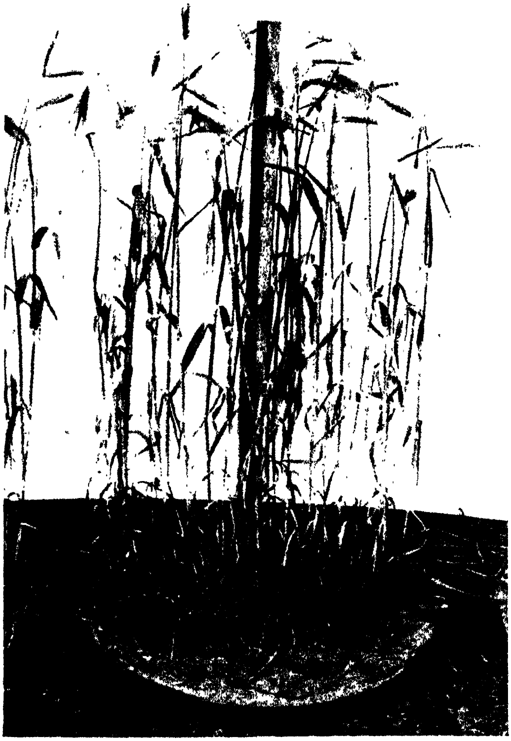

FIG. 9.—Mature crops: control, not fertilized

in the successive layers of the singly fertilized soils was progressively less from the surface foot downward, and greater in any soil doubly fertilized. The growth period of the plant, however, seems to affect the rate and significance of absorption more or less aside from the extent of root development.

Phosphorus depressed the yield. Larger quantities were absorbed where this salt occurred at deeper levels in addition to the surface soil, and the dry weight of tops was correspondingly depressed. The plants were fewer and smaller in almost every way. Reduction in yield varied almost directly in proportion to the amount of phosphorus absorbed. The average dry weight and average phosphorus content of straw of the controls, singly and doubly fertilized series, were respectively, 53.7, 43.5, and 28.4 gm., and 0.301, 0.423, and 0.565 per cent. In most cases, however, phosphorus increased the percentage of stalks bearing heads, while nitrates, except where they occurred below 2 feet, caused a decrease.

Nitrogen increased absolute yield only when present in the surface foot or in the surface foot as well as in lower levels. In either case the increase was brought about mainly through the straw and averaged greater under the second set of conditions. This effect through the straw was wrought simply by an enormous increase in the number of stalks. Nitrate depressed height growth, reduced the average weight per stalk, lowered the percentage of stalks bearing heads, and increased the total number of heads and their average length but little. It exerted only a slight influence upon the weight of the grain, yet the increase in the total number of stalks was sufficient to overcome these losses, and indeed to give increased totals. Unlike phosphorus, it lowered the ratio of the dry weight of grain to straw. This effect appeared where the plants absorbed more than 135 p.p.m. of NO_3, or where the straw contained above 1 per cent and the grain above 2.5 per cent of nitrogen. HELLRIEGEL and WILFARTH (12), working with sand cultures, obtained no further increase in the ratio of grain to dry matter of barley when the cultures were supplied with more than 168 mg. of nitrogen. HELLRIEGEL (13) also found that the percentage of grain to total dry weight of barley decreased when more than 113.6 mg. of P_2O_5 were supplied per pot.

The amount of nutrient removed from the soil is not the only factor affecting quality and quantity of yield. As regards nitrogen particularly, much seems to depend upon the time when the salts are available. Placing the fertilizer in the several containers in progressively deeper soil layers is really a means of varying the time in the life of the plant when the salt is available. And, since presumably roots absorb from deeper soil layers somewhat in proportion to the time they occupy these layers, it also varies the amount of fertilizer absorbed. Thus, as the time of nitrate absorption became progressively later in the life of the crop in the soil singly fertilized, so the number of stalks and heads, the nitrogen content of the straw, and, to a less degree, the nitrogen content of the grain decreased. Moreover, the same held true for the dry weight of both grain and straw and the average length of heads, where the fertilizer occurred above 2 feet. Where the nitrate occurred below 2 feet there was a relative increase in weight of grain and straw as well as length of heads, instead of a further decline. This occurred also in the doubly fertilized soils, where in addition gains were made in the nitrogen content of the straw, number and average length of stalks, number of heads, and percentage of stalks bearing heads. Thus applications of nitrate are most beneficial to the quantity and quality of the crop in the earliest and latest stages of root development and activity, although they are not entirely ineffective at certain intermediate stages.

The relation of the growth period of the plants to the effectiveness of the phosphorus was less marked, although very definite in some respects. As the contact of the roots with the fertilized layer was delayed, there was a progressive decrease in the number of stalks, dry weight of both grain and straw, and phosphorus content of grain.

GERICKE (8) applied sodium nitrate to spring and winter wheat, oats, and rye growing in soil in one gallon stone jars, at different growth periods. In the spring wheat and oats, the nitrogen or protein content increased continuously as the applications were made later and later in the life of the plant. Winter wheat and rye gave a like response only to applications made in the latest periods of growth. The barley in our experiment responded differently from these crops, in that the plants decreased in nitrogen content and in tillering as the availability of the nitrate was postponed through the mechanism provided in the singly fertilized series. GERICKE (9) confirmed his results regarding the relation he found between the time of application of nitrate salts and the resultant protein content of oat plants. He used a sandy soil deficient in nitrogen, and grew 7 plants in each one gallon container in a greenhouse. He found an increase in the number of stalks, but a decrease in their average height as the nitrate was applied later in the life of the plant. He believes the shorter stalks may be either a result of competition or due to the late production of tillers and their correspondingly shorter period for growth. Moreover, he found total dry weight and the weight of the grain to be greater when the nitrate was applied later in the life of the crop. In every respect these results are quite the converse of those set forth in this paper, and the reason for the difference seems to lie in the method of experimentation. It seems clear that cereal crops with roots which normally reach depths of 3–5 feet and spread laterally through a radius of 12 inches (WEAVER **19, 20**) might function quite differently when several plants were confined to 5.5 kg. of soil.

DAVIDSON and LE CLERC (3), working with wheat grown in outdoor plats, applied $NaNO_3$ at the surface at different periods of growth with the following results. Applications made early (plants 2 inches high) stimulated vegetative growth and consequently increased yield; applications made at the milk stage of the grain had no effect on either yield or quality; while applications at heading time gave a better quality of grain as to color and protein content, but the vegetative growth was unaffected. In 1922, DAVIDSON (4) again grew wheat in field plats of one square rod each and applied nitrates at different growth periods. The effectiveness of the nitrogen in increasing yield gradually decreased as the nitrate was applied later and later in the life of the plant. Its effectiveness, however, in increasing protein content of grain and percentage of grain to total dry weight of crop was increased. The data in table IV bear out these results except the effect upon the protein content of grain.

Burd (2) grew barley in containers with a surface area of 30×60 inches each and a depth of 18 inches. He concludes that the two elements (potassium and nitrogen) with which plant growth in general is most closely associated may approach or exceed their maxima at a comparatively early stage in the plant's development, that is, at the beginning of head formation; also that the mutual relations of soils and plants are such that it is generally desirable to have the large amounts of solutes incidental to relatively high concentrations in the soil solution at the commencement of the plant's growth cycle, but that it is unnecessary and may be undesirable to maintain this condition during the later stages of growth.

As regards nitrates, our results do not confirm this conclusion. Weaver (20) has shown that the roots of barley when grown in a column of soil of adequate depth do not reach the lowest levels of their penetration to any considerable extent, and consequently do little absorbing there until the plants are nearing the blossoming stage. Root activities during the critical periods of blossoming and the filling of the grain have been shown to be exceedingly important. Gile and Carrero (10), working with rice and corn where a part of the roots only were in contact with nitrogen or phosphorus in a complete solution, have shown that the smaller the portion of the roots in the complete solution the greater was the absorption of the elements per gram of roots.

Differences in the results obtained by various investigators on the use of nitrate fertilizers no doubt are due, at least in part, to the different crops and experimental methods employed. Moreover, it is possible that the effect of nitrates upon the development of a plant is determined in some measure by the portion of the root system in which the absorption occurs. Perhaps a smaller percentage of nitrate absorbed by the roots from the deeper soil levels actually reaches the above-ground parts, being used up in the metabolic processes of the roots themselves. If this is the case, the arrangement of the fertilized layers in the containers in this experiment would serve to make the results evident.

The fact that roots absorb nutrients at deep levels in the subsoil as well as from the surface layer should be given greater attention by all plant growers. The current idea that it is mainly the surface layer of soil that supplies the plant with nutrients and that the subsoil is the crop's reservoir for water, should give way to the fact that it is the whole soil mass pervaded by roots that determines root activities. Since not only the quantity of nutrients but also the time at which they are absorbed affect quantity and quality of yield, the amount of available nutrients in the subsoil is a matter of great importance. In fact, the adaptability of a soil for crops may be determined largely by the composition of the subsoil. The problem of getting the phosphatic and potassic fertilizers, which do not leach extensively into the deeper soil where they may be more efficient, is one with which students of fertilizer practices should be concerned. The time and quantity of the applications of nitrates, which leach freely into the deeper soil, in relation to rainfall and root depth is a field worthy of investigation. It is possible that tillage and cultivation practices can be modified so as to give in advance of the crop season a supply of nitrates which might leach into the deeper subsoil in time for their absorption at the most effective period in the development of the crop.

Summary

1. This investigation was undertaken to determine the effects of absorption of nitrates and phosphates from the subsoil on quantity and quality of yield of barley. Eighteen containers, 22 inches in diameter and 30 inches deep, were filled with soil and subsoil and placed in trenches in the field. Thirty barley plants were grown in each container; they were surrounded by a crop of barley, growing partly in the refilled trenches. The soil, except in the controls, was fertilized at various levels to 30 inches in depth, and in some cases at two levels, with either $NaNO_3$ or $CaH_4(PO_4)_2$. Wax seals, through which the roots easily penetrated, were used to prevent the movement of water and nutrient salts from the fertilized layer to the soil above or below.

2. The roots in the controls reached a depth of 30 inches, but nitrate fertilizer at any level tended to lessen root depth and greatly increased branching. Phosphates did not noticeably increase root development.

3. Nutrients were absorbed in large quantities at every level to 30 inches. Although the plants used the largest amount of salts

from the surface foot, they also took large additional quantities from the deeper levels when it was available.

4. Absorption of nutrients at levels below the surface foot affects materially the quantity and quality of the yield. It does not lose its additive effect even when the surface foot is abundantly supplied with a similar nutrient. Thus the chemical composition of the subsoil and the soil solution is very important.

5. Nitrates increased total dry weight when applied to the surface foot early in the life of the plant. This results from its effectiveness in promoting heavier tillering. It increased dry weight and also quality of grain still more when available at lower levels as well as in the surface foot. Phosphorus depressed yield, particularly that of the straw, somewhat in proportion to the amount absorbed.

6. Time of absorption is an important factor. The effects of the nutritive salts are most marked on both quantity and quality of yield early and late in the development of the plant, that is, when absorption is confined largely to the first foot of soil and the crop is tillering, and again when the younger portions of the longer roots are absorbing from the deeper levels at the time of heading. Thus an ample distribution of the deeper portion of the root system in a rich subsoil solution at the later critical period of growth is exceedingly important. Consequently, a knowledge of the development and extent of the roots of crop plants is of primary interest.

7. These experiments show the importance of the subsoil as a source of nutrients for crops, and the effects upon plant development. They emphasize the values to be gained by fertilizer practices which take the composition of the subsoil into account.

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MEIOSIS IN POLLEN MOTHER CELLS OF OENOTHERA FRANCISCANA SULFUREA

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(WITH PLATES XIV, XV)

Introduction

Oenothera franciscana sulfurea is an evening primrose with cream colored flowers, which arose in 1914 in the experimental gardens of B. M. DAVIS. An account of its appearance and subsequent history, for which I am indebted to Professor DAVIS, can be summarized briefly as follows:

1912. Plant 12.16*a biennis* was pollinated by *franciscana* B. (pollen supplied by H. H. BARTLETT, from his culture of *franciscana* in Washington).

1913. Culture 13.36, F_1 hybrids of *biennis* (12.16*a*)×*franciscana* B. This culture was fairly uniform (DAVIS 4), and resembled *franciscana* in most characteristics.

1914. Culture 14.56, from plant 13.36*a*, selfed. Germination was very poor (8 per cent), and the culture was small. The 51 plants which sent up flowering shoots were uniform and resembled closely the F_1 parent, 13.36*a*, except for one plant (14.56*w*), which differed from the rest in having cream colored ("white") flowers (DAVIS 5). This was the first *franciscana sulfurea*, and its history in selfed line has been as follows:

1916. Culture 16.23 from plant 14.56*w*. Contents of one capsule, forced to complete germination, gave 188 seedlings and 32 empties. Germination 85 per cent; 64 plants were grown to maturity, all white flowered.

1917. Culture 17.23, from plant 16.23*a*. Earth-sown; 12 plants were brought to maturity, all white flowered.

1920. Culture 20.23, from plant 17.23, I 3. Earth-sown; 25 plants were grown to maturity, all white flowered.

1921. Culture 21.23, from plant 20.23–6. Contents of one capsule forced to complete germination gave 354 seedlings and 49 empties. Germination 87.8 per cent; 23 plants were grown to maturity, all white flowered.

There is reason to believe that the *sulfurea* character has been inherited from the *biennis* side. *O. franciscana* has been grown now for eight generations, and has yet to show any sign of aberrancy. On the other hand, in *biennis*, as is well known, a small percentage of aberrant forms is to be expected, among which are a few *sulfurea* forms. STOMPS (7) found 4 *sulfurea* plants in 920 plants of *biennis* (0.413 per cent), and DE VRIES (8) found 27 in 8500 (0.317 per cent). The *sulfurea* character, therefore, may easily have come from the *biennis* parent. In most other visible characters the plant resembles *O. franciscana*, and for this reason it has been named *O. franciscana sulfurea.* It will be observed that, so far as the *sulfurea* character is concerned, this strain up to the present time has bred entirely true. Cultures have not been grown of sufficient size as yet, however, to warrant any statement regarding the genetical characteristics of the plant as a whole, beyond the fact that they have exhibited a certain amount of variation.

Material and methods

Material was collected during the summer of 1920 in the experimental gardens of Professor DAVIS at Ann Arbor, Michigan. Several killing fluids were used, but, as in the case of *O. franciscana*, it was found that by far the best results were obtained by fixing for three or four hours in one of ALLEN'S modifications of Bouin's solution, containing picric acid (saturated aqueous solution) 75 cc., formalin (commercial) 25 cc., glacial acetic acid 5 cc., chromic acid 1.5 gm., urea 2 gm. The material was imbedded in paraffin, cut at 8–10μ, and stained with iron-alum haematoxylin.

Description

Longitudinal sections of the anthers of *O. franciscana sulfurea* resemble very closely those of *O. franciscana* at corresponding stages. Archesporial and pollen mother cells of the two forms are alike as to size and arrangement, and their nuclei are also of the same size. The separation and rounding off of the pollen mother cells take place at the same time and in like manner. The resemblance is so close that, except for intra-nuclear differences at certain stages, sections from the two plants are practically indistinguishable.

ARCHESPORIUM

In the archesporial stage the resemblance extends even to the nuclear contents. There is the same delicate and copious reticulum seen as in *O. franciscana* (CLELAND 1), irregularly roughened and thickened with chromatin aggregations of varying size and shape, and in intimate contact with the nucleoli, of which one is usually much larger than the rest, if more than one is present. There is also the same absence of parallelism, the threads showing little if any tendency to pair. This condition is not only true of the archesporial resting stage, but of later stages as well. As the nucleus begins to approach the heterotypic prophase, some of the threads seem to shorten and thicken, which results in the stretching and attenuation of others. As a consequence, the meshes of the network become more irregular in size, and occasionally threads are brought into parallel relation with one another, a condition which might be interpreted as the parallelism of homologous threads. Such a large proportion of the threads remain undoubtedly single, however, and the occasional parallelisms are so obviously a result of the contractions and irregular amalgamation of threads which finally lead to the formation of the "open spireme," that there does not seem to be any reason for believing that a fusion takes place at this stage, either of homologous parts of two different spiremes, or of the split halves of the same spireme.

HETEROTYPIC PROPHASE

SYNIZESIS.—Early and mid-synizesis stages resemble exactly corresponding stages in *O. franciscana.* The reticulum contracts in the same way to one side of the nucleus. This is usually the side which is nearest the periphery of the cell, which suggests the possibility that synizesis is an artifact, due to the influence of the fixing fluid. The nucleolus also leaves its original position in the interior and moves to the same side of the nucleus, where, buried in the reticulum, it becomes plastered up against the periphery as a somewhat flattened, cushion-shaped, black staining body. The contracted mass of threads becomes so compact that it is impossible to see the individual threads, except along the periphery. Thin tangential sections and a study of the lateral surfaces of the knot are necessary in determining the situation at this stage. Most of the threads have become exceedingly tortuous and much wrinkled, as though they had shrunken. They are very unequal in diameter, some seeming to be increasing in importance and others diminishing, as described for *O. franciscana*. The spaces between the threads in such a contracted knot are very small of course, but there is no indication that threads parallel one another for any distance. There is rather a dense tangle of threads, which run in all directions, seemingly without order or system.

As synizesis proceeds, the number of threads decreases by the transfer of material composing certain threads into others, and by the fusion, in various ways, of adjacent parts of the reticulum. The surviving threads become more prominent, and the meshes in the network become larger, even though the synizetic knot as a whole does not increase in size. Little by little these threads begin to be distinguishable in the knot when examined as a whole, at first rough and unequal in diameter, but later becoming smoother and more uniform. As the threads become more prominent, the nucleoli flatten out more and more, the large one often assuming the form of a horseshoe or ring, or even fragmenting.

Thus far, the development of the nucleus has been so like that of *O. franciscana* that figures illustrating the latter will do equally well for this plant (CLELAND 1, figs. 1–8). The manner of the unfolding of the knot, however, differs in detail in the two plants. In *O. franciscana* it was found that the threads nearest the nucleolus became gorged and swollen with chromatin, which passed out from the latter, and gradually diffused throughout the reticulum. The thread system, with its chromatin content thus augmented, unfolded and became a diffuse and open reticulate spireme, to a large extent filling the nucleus, a typical "open spireme." In *O. franciscana sulfurea* the chromatin contents of the nucleolus pass into the reticulum also, but in a slightly different way. The flow seems for the most part to be confined to one or two threads which lead directly from the nucleolus to the center of the knot (figs. 1–3).

These become gorged and greatly swollen. Chromatin material passes along these to the center of the reticulum, and from there into the other threads. At the same time, the synizetic knot begins to unfold slowly. The main part of the spireme, composed by this time of threads which for the most part are prominent enough to be visible, leaves the region of the periphery and the nucleolus, and comes to lie in the center of the nucleus, leaving only a few strands bending and twisting in an open network between itself and the nucleolus. Through this openly reticulate portion pass the swollen threads, forming a broad, black staining, and straight band. There is no question in my mind that material is flowing through these threads into the reticulum. The attachment of the nucleolus to the broad band or bands is unmistakable, and a study of the nucleolus shows clearly that it is being emptied. At first it is uniformly black staining (figs. 1, 2). As the threads begin to swell and stain black, however, the edges of the nucleolus commence to lose their staining qualities. The black staining portion gradually becomes smaller and smaller, and more and more confined to the region around the exit (fig. 3). Soon the endonucleolus appears, freed from its covering of chromatin, a black staining little body in the almost unstained and empty looking portion of the nucleolus. The black color finally disappears entirely from the nucleolus and it is free from chromatin (fig. 4). Meanwhile the threads of the reticulum which are connected with the other end of the broad band have become swollen and black also, as the chromatin is passed out into the rest of the network. Gradually the chromatin diffuses through the reticulum, until at last it is distributed more or less evenly throughout the whole system.

OPEN SPIREME.—The open spireme (fig. 4) on the whole is not as open as it is in *O. franciscana*, nor is it diffused through as large a portion of the nucleus. Throughout the entire stage there is usually a region toward the center where the threads are crowded together into a rather dense tangle, and the looser portions of the reticulum as a rule are confined to a fairly small area. The threads fail to show any indication of a longitudinal split or of parallelism, even in those nuclei which have been very much destained. The presence of chromomeres can generally be demonstrated. Sometimes they are very prominent, strung along the spireme like beads on a string. At other times they are more or less crowded together, so that their individuality is partially or entirely obscured.

SECOND CONTRACTION.—As the second contraction period draws near, the central, more densely tangled region, which has never entirely disappeared, becomes more prominent, for all of the threads begin to contract, commencing at the center. At the same time, the peripheral portions of the reticulum become thrown into more or less clearly defined loops of varying size and appearance (figs. 5, 6). Contraction continues for some time, and, as the threads in the central region become more and more swollen and closely packed, they gradually form a roughly spherical aggregation, so dense that its structure cannot longer be determined (fig. 7). At the same time the peripheral loops shrink in size. In some cases this means a corresponding increase in the diameter of the threads making up the loops, but more often it seems that most of the material composing them flows or migrates into the more central portion of the reticulum, so that the loops themselves, although smaller, are composed of no thicker threads than they were before contraction began. Occasionally the loops become greatly narrowed, the two sides approaching one another until in some cases they become contiguous. As these loops radiate out from the central knot, it sometimes happens that a pair of them may lie so near to each other that at first sight one is tempted to consider them the separated halves of a larger loop, each half consisting of two parallel threads, a condition which would strongly suggest parasynapsis. A careful study of the way in which these structures are formed in the earlier stages of contraction, however, makes it clear that they are individual loops, the unsplit sides of which have approximated (fig. 6).

Contraction continues until in many cases the loops practically disappear into the central knot. In a considerable proportion of the cells, however, the loops do not entirely disappear. One can find nuclei in all stages of second contraction, in which the outer ends of the loops can be seen protruding beyond the edge of the knot (fig. 7). At first the component threads are thin. Contraction has not caused them to increase in diameter in many cases, because their contents seem to have largely been removed toward the interior. After the period of maximum contraction has been reached, however, material begins to flow back again. The threads making up the loops increase in thickness, and the loops become more prominent as a result.

FINAL PROPHASE STAGES.—The compact second contraction knot begins to loosen as the nucleus passes into the stage generally known as diakinesis, and little by little resolves itself into a spireme, now greatly thickened, of which the loops which were usually observed during the contraction period constitute a part (figs. 8, 9). At first greatly tangled, especially toward the center, the spireme in a short time becomes more or less completely unfolded, revealing an arrangement of the chromosomes at the same time interesting and unusual (figs. 10–13). In the first place it will be observed that the spireme does not, as in most plants and animals, break up into pairs of homologous chromosomes which separate and stand apart during diakinesis, but remains intact and unbroken. In the second place, it can easily be seen that the chromosomes are arranged end to end in the spireme. They are somewhat spindle-shaped, thickest in the middle and tapering at the ends, and are usually attached only by a thin thread. Consequently they are clearly distinguishable and can be counted with ease, the diploid number always being found. There can be no question that at this period the spireme is univalent, consisting of only one row of chromosomes, which are attached end to end, or telosynaptically.

The appearance at this time is interesting for a third reason. The chromosomes are always arranged in such a way that the spireme is formed into a definite and characteristic figure, which is present in all nuclei at this stage. It will be remembered that in *O. franciscana* also the chromosomes are joined together in a very definite and constant manner in diakinesis. In the latter plant four of them are attached end to end to form a closed circle, to which are linked three smaller rings, each consisting of two chromosomes; and to two of these there is linked in turn another ring, made up also of two chromosomes. In *O. franciscana sulfurea* the chromosome arrangement is as definite and constant as it is in *O. franciscana*, but it is different, being somewhat simpler, and in some ways a more striking grouping. Of the fourteen chromosomes, twelve are joined end to end to form a large closed circle, and to this circle is linked one small ring, composed of two homologous chromosomes. The circle of twelve is usually too large to be accommodated in the nucleus without a certain amount of twisting and turning, but there is not the slightest difficulty in most cases in following along the chain from a given point back to the same point again, and in determining that the number of chromosomes making up the circle is twelve. The individual chromosomes at this time are rather large, and usually stand apart distinctly. Their surfaces are uneven and soft looking, and one gets the impression that probably they are quite spongy in texture.

It is generally necessary to have the whole nucleus in order to see the ring in an unbroken and complete condition, and when sections are cut at 8–10 μ there will not be a very large proportion of entire nuclei. Practically every whole nucleus examined at this stage, however, has shown the unbroken circle clearly, and thirty-seven very clear cases have been recorded. In addition, a large number of incomplete nuclei show from eight to ten chromosomes in a string, with the separate ring often appearing, so there can be no doubt that a circle of twelve chromosomes, plus another pair, constitute the normal arrangement at this time.

The end to end arrangement of so many chromosomes confirms very strikingly the conclusion that telosynapsis is present here. Were there but six or seven chromosomes at the most so attached, we might conclude that in the earlier prophase stages the chromosomes had formed a bivalent spireme, the halves of which had subsequently separated. When we find twelve of the fourteen chromosomes placed end to end, however, it can hardly be doubted that the spireme has been univalent from its beginning, and has failed to segment transversely into its bivalent components.

The constantly uniform placing of the chromosomes also tends to emphasize the suggestion previously made (1) that the chromosomes may be distributed in earlier stages of the heterotypic prophase, and perhaps even in somatic nuclei, according to a definite system, and not merely in a chance manner. We now have two plants which just previous to the heterotypic metaphase show an arrangement of chromosomes characteristic and uniform for the form, which may point toward a more perfect orderliness and greater uniformity in nuclear structure than has hitherto been realized.

The nucleolus behaves during this period exactly as did that of *O. franciscana*. It is found plastered up against the nuclear membrane, where it gradually melts away and disappears. The little endonucleolus is also present. This arrangement of chromosomes persists throughout the entire period. The twelve chromosomes making up the large ring remain attached. The small ring, however, may separate from the large one and lie independently in the nucleus (figs. 11, 12). During the latter part of this stage, spindle fibers begin to appear, closely investing the nuclear membrane, and when the latter melts away, they penetrate rapidly into the region of the nucleus. Meanwhile the chromosomes shrink greatly, until they are scarcely one-fourth the size previously presented (figs. 14, 15).

HETEROTYPIC METAPHASE

At first the spindle is multipolar, but soon it becomes bipolar. Enmeshed in the maze of spindle fibers are the chromosomes, still attached to one another, and lying in an irregularly tortuous cluster (fig. 15). The bivalent pair, if it has not already separated from the circle of twelve, does so some time during this period.

In view of the fact that the chromosomes are still joined together, and the bivalent pairs do not act separately as independent units, we should not expect to find in this plant that regularity of chromosome position in metaphase, and separation in anaphase, so characteristic of *O. franciscana*, in common with most plants and animals. It is surprising, therefore, to what degree normal distribution of the chromosomes to the poles is found in spite of what we should suppose would be handicaps to such behavior. The chromosome chain lies irregularly contorted and twisted in the midst of the spindle fibers. We should expect the chromosomes to become attached to whatever fibers approach them most nearly, irrespective of from what pole these might emanate. Even if the chain were not so twisted, but lay horizontally across the cell, so that the chromosomes were all equidistant from the two poles, it is not easy to see what would prevent such a chance attachment. When chromosomes, instead of all being joined together, are paired in diakinesis, as they are in most organisms, it is natural that the members of a pair should become attached to the fibers leading to different poles, for in practically every case one chromosome lies nearer one pole, and the other nearer the other pole. There is every reason to suppose, however, that it makes no difference which chromosome of a pair is nearer a given pole; the position of the two chromosomes of a pair, with reference to the poles, is regulated entirely by chance. If, then, in the cells of typical plants, chance determines which chromosomes of a bivalent pair shall become attached to fibers going to a certain pole, there is little reason to believe that the situation in *O. franciscana sulfurea* is different. In this plant also it seems to be entirely a matter of chance which pole a given chromosome is nearest, as it lies attached to its neighbors in the winding twisted chain, and we should expect, therefore, an entirely unregulated attachment to the fibers, and distribution to the poles.

It is surprising to find, therefore, that this process takes place in a markedly regular manner. In most nuclei the chromosomes in the circle of twelve alternate in their attachment (figs. 16, 18). Fibers from one pole will become attached to the first, third, fifth, etc., chromosome of the chain, and those from the other pole to the second, fourth, sixth, etc. They are usually fastened to the middle of the chromosome. When the fibers begin to contract, the equal pull from the two poles brings the circle of twelve into a horizontal position across the cell. The chromosomes, still attached to one another at their ends, but pulled in opposite directions by the fibers fastened to their mid-regions, become *V*-shaped, so that the chain as a whole takes on a regular zigzag appearance. Meanwhile the independent bivalent pair of chromosomes acts in a perfectly normal manner.

The chromosomes continue their attachment throughout metaphase. The spindle fibers appear to exert a pull upon the chromosomes, and they are stretched farther and farther apart (fig. 18). Their ends grow more and more attenuated, until at last they can stand the strain no longer, and snap. The chromosomes are thus set free and quickly pass to the poles. It is not until anaphase, therefore, that the circle of twelve chromosomes is finally broken up.

While in general the chromosomes are distributed equally and regularly to the poles, in some cases the distribution is unequal (figs. 17, 20). Occasionally a chromosome will become attached to fibers from the wrong pole, so that two adjacent chromosomes are pulled in the same direction. This results in eight chromosomes going to one pole and six to the other. I have tried to estimate the proportion of cases in which the chromosomes are thus unequally distributed, by studying the cells during late interkinesis (figs. 22, 23). At this stage the chromosomes stand apart clearly, and it is possible to tell accurately in a large majority of cells whether seven went into each daughter nucleus, or some other number. At this stage 623 cells were complete enough to be certain of the chromosome number. Of these, no cell was found in which the number going to one pole exceeded eight. On the various slides studied, the proportion of cells in which the chromosomes had been irregularly distributed, eight going to one pole and six to the other, ranged from 11 to 30 per cent. The average for all the cells counted was 16 per cent. This probably represents fairly accurately the average percentage of irregular distribution in this plant, a strikingly small percentage when one considers the way in which the chromosomes are united during metaphase.

ANAPHASE TO INTERKINESIS

Once separated, the chromosomes pass rapidly to the poles. In most cases they are in the form of short, fat *V*'s, but as yet show no signs of a longitudinal split (fig. 19). At the poles they are grouped closely together (fig. 21), and are soon inclosed by a nuclear membrane. A central chromosome is usually found, surrounded by six peripheral ones, although this arrangement is upset when six or eight are present instead of seven. From now on the pollen mother cells are developed, and the pollen grains are formed, much as in *O. franciscana*. As the daughter nuclei grow, the chromosomes form slight attachments to one another. They become irregular in outline, but their identity is rarely lost. Occasionally there are signs in early interkinesis of the split in the chromosomes preparatory to the homoeotypic division, but as a rule this does not appear until later, when the chromosomes have lost all contact with one another. The fact that the split has taken place finally becomes evident when the two grand-daughter chromosomes separate along most of their length, and remaining in contact only at the middle, twist around in such a way as to form a maltese cross (figs. 22, 23). Nucleoli are developed *de novo* in contact with the chromosomes, but do not reach a large size. In all respects, therefore, interkinesis in this plant is like that in *O. franciscana*.

HOMOEOTYPIC MITOSIS

No wall is formed between the daughter nuclei. As the time for the homoeotypic metaphase approaches they begin to shrink, and spindle fibers make their appearance. The two nuclear membranes disappear simultaneously, and the chromosomes are enmeshed in multipolar spindles, which soon become bipolar. By this time the chromosomes have lost their *X* shape, the two halves of each having condensed into a pair of small, almost spherical bodies, which lie more or less closely appressed (fig. 24). The homoeotypic metaphase differs markedly from the heterotypic because of the rounded rather than *V* shape of the chromosomes, and by the fact that these are in pairs, which are not connected with one another. The two metaphase figures lie in opposite sides of the cell, sometimes in one plane, but often at right angles to each other, or at least in different planes (figs. 25, 26).

The grand-daughter nuclei are constituted as were the daughter nuclei, the six, seven, or eight chromosomes being very close together at first, but separating as the nucleus grows. The nucleus passes rapidly into a resting condition, the process being similar to that already described for *O. franciscana*. The chromosomes do not split, but send out delicate strands which meet and fuse, forming an open network upon which is distributed in irregular fashion some of the chromatin. When the resting condition is finally attained, the chromosomes have so amalgamated that their individuality is completely lost (CLELAND 1, figs. 36–38).

The four nuclei lie equidistant from one another in the cell. The more or less prominent sets of spindle fibers developing between them in *O. franciscana* have not been noticed in this plant. The walls separating the spores are not developed until the nuclei are completely at rest; then they are formed after the manner described for *O. franciscana*. All of the walls are formed at the same time. The first trace of their appearance consists in delicate cleavage lines, appearing at the periphery of the protoplast midway between the nuclei. These run quickly into the interior, meet, and soon grow into much wider walls. The process is probably very rapid, as early stages seem to be rare. The wall thickens much more rapidly toward the outside than farther in, so that the protoplasts are separated more widely at the exterior. No cell plates have been found, and it seems quite certain that the walls are formed by very rapid furrowing, which proceeds from without inward.

Discussion

In some respects the cytological development of the pollen grains of *O. franciscana sulfurea* bears a strong resemblance to that in *O. franciscana* (CLELAND 1), to which it is closely related, emphasizing the suggestions and confirming the conclusions drawn from a study of the latter. In certain particulars, however, the two plants show differences in development which are of interest and importance.

CYTOLOGICAL

From the cytological standpoint, the situation in *O. franciscana* was of interest principally for two reasons: (1) it presented a clear case of telosynapsis; (2) the chromosomes in diakinesis showed a constant and definite arrangement, which was both striking in appearance and suggestive of a higher degree of order in the nucleus than has perhaps generally been imagined. Both of these points are clearly emphasized in *O. franciscana sulfurea* also.

TELOSYNAPSIS.—If anything, *O. franciscana sulfurea* shows telosynapsis even more clearly than does *O. franciscana*. In early heterotypic stages, both plants are alike in displaying very little that could be used as evidence for the synaptic pairing of two univalent spiremes to form a bivalent one, or for that matter, for the presence of a longitudinal split in a univalent spireme. This is true of pre-synizetic stages as well as of all subsequent ones. In addition to negative evidence of this sort, there is positive proof that chromosomes are placed end to end in a univalent thread system. The unsplit spireme of the "open spireme" stage forms itself into loops. These loops can be observed in all stages of second contraction, and when the unfolding process occurs, it can be seen that from them the chromosomes have been formed without any splitting having occurred. The chromosomes are but segments of the whole spireme, therefore, and must have been placed end to end during the spireme stages.

O. franciscana sulfurea also furnishes convincing evidence of the presence of telosynapsis in the way the chromosomes are arranged after this unfolding takes place. At this stage, twelve of the fourteen chromosomes are found to be attached end to end in all nuclei, and form a large closed circle. Such a condition makes it difficult to accept any interpretation other than the telosynaptic one.

CHROMOSOME ARRANGEMENT.—An arrangement of the chromosomes in diakinesis into a definite figure, such as was seen in *O. franciscana*, is interesting. It becomes doubly so when we learn that a nearly related plant also shows a definite chromosome grouping, especially when it appears that the grouping in this plant is a different one from that seen in *O. franciscana*, namely, twelve chromosomes arranged in a closed circle, to which is linked a ring consisting of two chromosomes. It emphasizes the suggestion made (1) that perhaps this definite arrangement may be carried back into much earlier stages of the heterotypic prophase, or even into pre-existing cells. In most plants and animals, it is not possible to obtain any evidence regarding chromosome arrangement in this way, since in diakinesis the bivalent chromosomes entirely separate from one another. Thanks to the tendency of the chromosomes to cling together in the species of *Oenothera*, we are enabled to get what may be a hint regarding nuclear construction in a much wider range of forms. It may be that even the position that chromosomes assume with reference to one another in the nucleus may be determined according to fixed rules.

It may be objected that the arrangement here seen represents nothing more than the inability of all but two of the chromosomes to pair, which results in the remaining ones retaining their attachment, and forming a spireme. The uniform presence of the circle of twelve chromosomes, however, strongly suggests an orderly arrangement of the chromosomes among themselves. Furthermore, although all of the chromosomes of the circle look alike, and one cannot be distinguished from another, there is reason to believe that the chromosomes are not scattered promiscuously in the spireme, but are definitely arranged. In the first place, attachment, or at least close approximation of the homologous chromosomes, is the usual condition during diakinesis in plants and animals, whether they have been brought into this relationship telosynaptically or parasynaptically; and we should naturally expect to find in this plant that even though the chromosomes still maintain their position in the spireme, they nevertheless are so arranged that the homologous ones are closely associated. Such a conclusion is also supported by the following considerations.

One can permute the total number of ways in which twelve chromosomes in a circle can be arranged; and, bearing in mind that adjacent chromosomes generally pass to opposite poles in this plant, it is also possible to determine the number of ways in which they can be so arranged that the members of the six pairs will in all cases separate and go to opposite poles. Such a result will be obtained only when homologous chromosomes are adjacent, or are separated from one another by intervals of two or four places. For instance, if chromosome *a* occupies position no. 1, its homologue a^1 must be in positions no. 2, 4, 6, 8, 10, or 12 in order to be carried to the opposite pole. The result of such computation shows that the ratio of the total number of possible arrangements to those in which the homologous chromosomes are so placed that all of them will separate in the reduction division is 462 : 1.[1] If, therefore, the chromosomes are scattered at random in the circle of twelve, on the average only one cell in 463 will experience an entirely normal reduction division. What is in effect a form of nondisjunction will occur in the other 462 cases, for both members of at least one pair will go to the same pole. In view of the fact that

[1] The twelve chromosomes, acting as units, can be arranged in $\frac{|\underline{11}}{2}$ ways. They can be arranged so that the members of all of the six pairs separate in $\frac{|\underline{6} \cdot |\underline{5}}{2}$ ways.

about 50 per cent of the pollen grains in this plant are apparently functional, that the percentage of seed fertility is high, and that the resulting plants are probably in general fairly uniform, it certainly does not seem probable that the proportion of typical reduction divisions is so infinitesimal. We conclude, therefore, that the chromosomes are arranged in a definite way. The simplest arrangement, and the one most to be expected from our knowledge of reduction divisions in general, is that in which the homologous chromosomes lie side by side; and I am inclined to believe that such is the chromosome arrangement in *O. franciscana sulfurea.*

GENETICAL

O. franciscana sulfurea differs from *O. franciscana* in being of known hybrid origin. It appeared in the F_2 generation of the cross *O. biennis* × *franciscana.* The F_1 generation resulting from this cross was strongly patroclinous, and when selfed gave in the F_2 a culture which in general tended to resemble the F_1. One plant appeared, however, which had cream-yellow ("white") flowers, the first *O. franciscana sulfurea.* This plant has been carried on in selfed line for four generations. It has bred true in respect to the color of its flowers, but beyond this it is not possible to say much regarding the nature of the plant from the standpoint of breeding. It undoubtedly exhibits a considerable amount of variation, but the cultures thus far have been so small that a study of this variation has not been possible. There are no breeding data available as yet, therefore, which will answer the question as to whether the strain is pure or hybrid in nature. Regarding the fertility of its pollen, only one test has been made, two plants being examined in 1917. These showed about 50 per cent of abortive pollen grains, which would admit of the possible destruction of large classes of gametes, and the consequent maintenance of uniform cultures in what is really a hybrid stock. On the other hand, the percentage of seed germination is high, so that while *O. franciscana* fulfils closely the tests of a pure species, the situation in *O. franciscana sulfurea* must be regarded as less certain, since its origin and the high percentage of pollen abortion both suggest a possible heterozygosity.

From the genetical standpoint, the chief interest in the cytological work on *O. franciscana* lay in the fact that it showed a perfect regularity of chromosome pairing in diakinesis and the heterotypic metaphase, a situation unusual in species of *Oenothera*. This fact, added to the other evidence, points to the probability that *O. franciscana* B, at least to a large degree, is a genetically pure race.

It is interesting to find that *O. franciscana sulfurea*, although resembling *O. franciscana* closely in most vegetative characters, differs markedly from it in this feature, displaying an almost entire absence of chromosome pairing during diakinesis. One pair only is formed; the other twelve chromosomes are strung together end to end, and form a large closed circle, which lasts until the end of metaphase. The failure to pair is not characteristic of *O. franciscana*, or of other stable species of plants, but is found in most of the unstable species of *Oenothera*, and is also characteristic of interspecific hybrids in general.

If we compare figs. 10–13 with the figures presented by GATES (6) for *O. rubrinervis*, and by DAVIS (2) for *O. biennis* (an American strain), *Lamarckiana*, and *O. gigas* (3), a striking resemblance will be noted. The chromosomes in these plants for the most part are joined together end to end throughout the stage generally known as diakinesis, in a manner much like that seen in *O. franciscana sulfurea*, the failure to show definite pairing being in general characteristic of them. *O. franciscana sulfurea* belongs with these plants, therefore, rather than with *O. franciscana*, in respect to this important cytological peculiarity, the failure of homologous chromosomes to pair in diakinesis.

The regularity of chromosome pairing in *O. franciscana* probably indicates that this plant is largely homozygous in nature. In marked contrast with this situation is the irregularity of *O. franciscana sulfurea*, a condition which may be the result of incompatibility on the part of homologous chromosomes, which would therefore mean that the plant is largely heterozygous. The cytology of other forms not included in the *Lamarckiana* series should be studied, however, and especially of *O. biennis*, one of the ancestral forms of this plant, before this position is definitely assumed as a hypothesis. While the failure of chromosomes to pair may be said to suggest the possibility of hybridity, therefore, I do not believe that the point is in any sense proved as yet.

Summary

1. *O. franciscana sulfurea* is an evening primrose with cream-yellow flowers, which appeared first in 1914, in the F_2 generation of the cross *O. biennis* × *O. franciscana* B. It has been grown for four seasons in small cultures, and has bred entirely true for the color of its flowers. Except for this characteristic, it resembles *O. franciscana* fairly closely.

2. Archesporial nuclei possess a delicate and copious reticulum of distinct threads, irregularly roughened and thickened with chromatin aggregations, but displaying no parallelism. The identity of the individual chromosomes is entirely lost.

3. The approach of the heterotypic prophase is indicated by an increasing irregularity in the diameter of the threads and of the meshes. This process appears to be the result of the passage of material composing certain threads into others, causing the gradual disappearance of some and the augmentation of others. The threads remain persistently single, only occasional instances of parallelism being found, to which no far reaching significance can be attached.

4. During synizesis, this process continues until the number of threads and meshes has become decreased, and the remaining threads are much thicker than before. The synizetic knot is usually found pressed against the nuclear membrane on the side which is nearest the periphery of the cell. Its position, therefore, seems to be due to the influence of the fixing fluid, which suggests that the synizetic contraction itself is a result of the same cause.

5. Up to this point, the development of the pollen mother cells has paralleled exactly that seen in *O. franciscana*. From now on, however, differences begin to appear, some of which are striking.

6. As the nucleus passes into the "open spireme" stage, the synizetic knot gradually assumes a central position, and begins to loosen. The nucleolus remains plastered against the nuclear membrane. It is filled with an intensely staining substance, probably

chromatin. This material passes into the reticulum by way of one or two prominent threads, which attach the nucleolus to the spireme, and is gradually spread somewhat evenly over the threads as they form the open spireme. The emptying of the nucleolus exposes an intensely staining little endonucleolus.

7. The spireme does not become so evenly distributed through the nucleus as it does in *O. franciscana*. The central portion of the reticulum remains somewhat densely tangled throughout the whole of the open spireme stage. There is no indication whatever of parallel threads, or of a longitudinal split in the spireme at this stage. Chromomeres are clearly present in many cases.

8. The reticulum begins to contract in the center, at the close of the open spireme stage. The peripheral parts are thrown into prominent loops. Condensation may go on to the point where in second contraction nothing appears in the nucleus but an irregularly rounded chromatin mass. More generally, however, the loops do not contract sufficiently to entirely disappear into the central region, and their identity can be traced throughout the whole period.

9. When the second contraction knot unfolds, it can be seen that these loops are a part of a long chain of chromosomes, which are attached end to end. When completely unfolded, the fourteen chromosomes are found to be arranged into a closed circle, consisting of twelve chromosomes, placed end to end; and the remaining two form a pair which at first is linked around the larger chain, but later becomes separated. This arrangement is found in all pollen mother cells at this stage. The nucleolus gradually melts away during this period.

10. The circle of twelve chromosomes is still unbroken upon the disappearance of the nuclear membrane. A multipolar spindle is formed, becoming bipolar. The circle of twelve is brought to the equatorial plate and the chromosomes are so arranged that alternate chromosomes go to the same pole. The remaining pair of chromosomes behaves in the usual manner.

11. The circle of twelve does not break up until early anaphase. Chromosomes are generally distributed equally. In a varying

percentage of cases (averaging 16 per cent) the arrangement of chromosomes in the circle of twelve is upset in metaphase, resulting in six chromosomes going to one pole and eight to the other.

12. Daughter nuclei are reconstituted after the manner described for *O. franciscana* (1), and the subsequent development almost exactly parallels that found in the latter plant. The chromosomes retain their individuality throughout interkinesis. Late in this period a longitudinal split appears, and the two halves of the chromosomes swing apart at right angles, so that each chromosome resembles a maltese cross.

13. The two daughter nuclei pass through the homoeotypic mitosis simultaneously, and are separated into individual cells at the same time. Walls are formed by rapid furrowing, which takes place from without inward. No cell plates are formed.

14. Nuclei with six or eight chromosomes, instead of the usual seven, develop normally as far as they can be traced.

15. The chromosomes seem to be arranged telosynaptically in this plant.

16. The constant grouping of the chromosomes in late prophase into two groups of twelve and two, as well as the generally normal distribution in the first anaphase, suggests the possibility that chromosomes have definite positions with reference to one another in nuclei, and are not scattered promiscuously, and without order.

17. The failure of all but one pair of homologous chromosomes to actually pair in diakinesis suggests the possibility that *O. franciscana sulfurea* is to a large extent heterozygous.

In conclusion, I wish to express my thanks to Professor B. M. DAVIS, for allowing me to collect from his cultures the material upon which this paper is based, and for furnishing the data regarding the history of *O. franciscana sulfurea*, as well as for his continued interest in the work. I am also indebted to the Marine Biological Laboratory, at Woods Hole, Massachusetts, for supplying me with facilities for carrying on the work during the summer of 1922.

GOUCHER COLLEGE
BALTIMORE, MD.

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EXPLANATION OF PLATES XIV, XV

All figures were drawn with the aid of a camera lucida, using a Spencer compound binocular microscope, and a Bausch and Lomb 1–12 apochromatic objective with 25X compensating ocular. They have been reduced one-fourth in reproduction; present magnification approximately 2800 diameters.

PLATE XIV

Fig. 1.—Late synizesis: chromatin material beginning to pass from the nucleolus into reticulum; latter much denser than here represented, the drawing being confined as nearly as possible to one level.

Fig. 2.—Slightly later stage.

Fig. 3.—Emptying of nucleolus and passage of material into spireme.

Fig. 4.—"Open spireme" stage.

Fig. 5.—Early second contraction, which begins at center, and peripheral portion thrown into loops; central region may not be as formless and solid as drawing indicates.

Fig. 6.—Later stage; narrow loops.

Fig. 7.—Second contraction about time of maximum contraction; endonucleolus shows clearly.

Fig. 8.—Second contraction knot begins to unfold.

Fig. 9.—Later stage, showing chromosomes arranged telosynaptically into spireme.

Figs. 10–13.—Spireme completely unfolded, showing arrangement of chromosomes, twelve of them forming large closed circle, and other two a small ring; in figs. 10 and 13, ring still linked to larger group; in figs. 11 and 12 it has become detached; nucleolus shown in process of melting away in figs. 11–13.

FIGS. 14, 15.—Multipolar spindle; chromosomes still attached.

PLATE XV

FIG. 16.—Typical heterotypic metaphase, showing only the group of twelve chromosomes, still attached, and arranged in regular zigzag fashion, alternate chromosomes headed for same pole.

FIG. 17.—An atypical heterotypic metaphase, showing irregularity in zigzag arrangement, which may result in six chromosomes going to one pole and eight to other.

FIG. 18.—Early anaphase, chromosomes widely separated, but still clinging together; all chromosomes shown, and arrangement regular.

FIG. 19.—Late anaphase, polar view; plates *a* and *b* from same cell, showing unequal distribution.

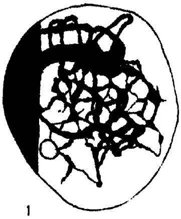
FIG. 20.—Part of spireme in heterotypic metaphase, showing irregularity in zigzag arrangement.

FIG. 21.—First telophase, just before formation of nuclear membrane.

FIGS. 22, 23.—Late interkinesis, showing split chromosomes, and formation of new nucleoli; one nucleus shows seven, the other eight chromosomes; the two figures from different cells.

FIG. 24.—Homoeotypic metaphase.

FIGS. 25, 26.—Homoeotypic anaphase: fig. 25 shows eight chromosomes in one figure and six in other; fig. 26 shows seven chromosomes in each.



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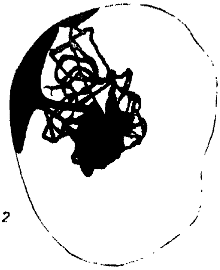

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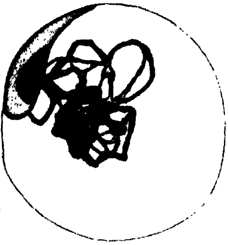

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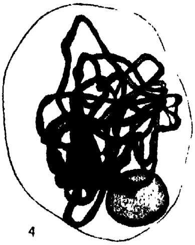

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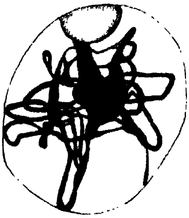

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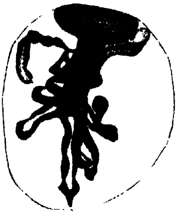

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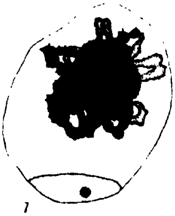

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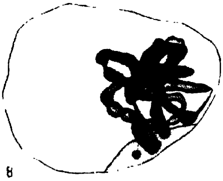

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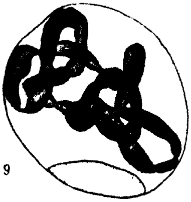

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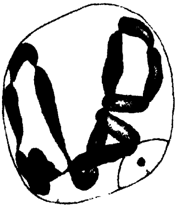

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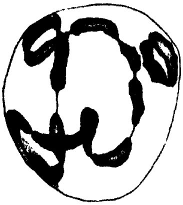

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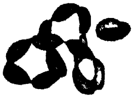

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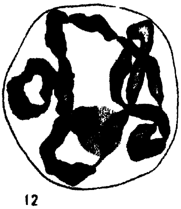

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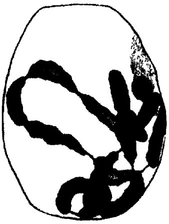

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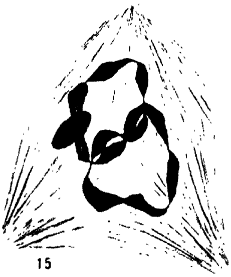

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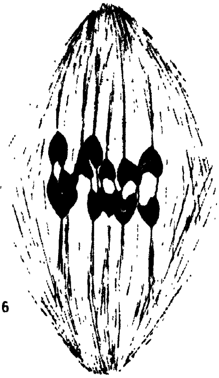
CLELAND on OENOTHERA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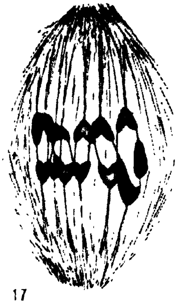
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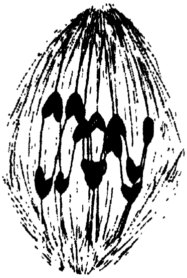
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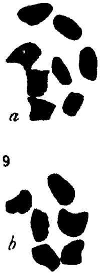

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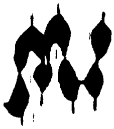
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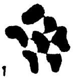
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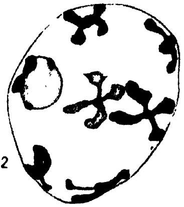
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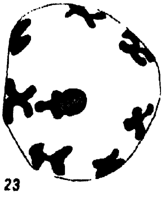
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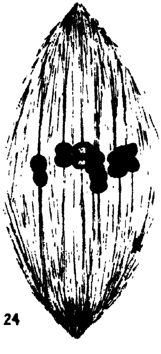
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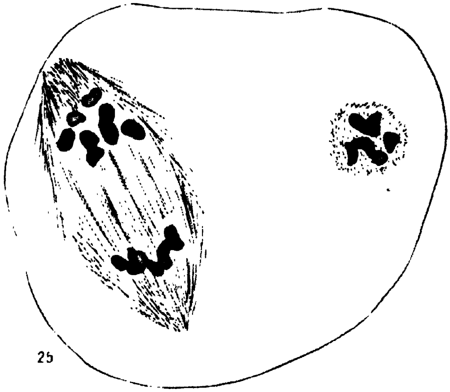
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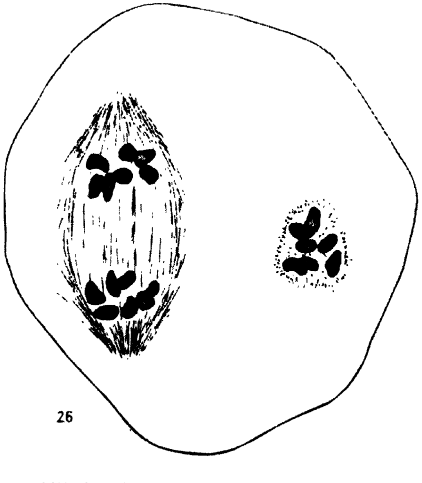
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CLELAND on OENOTHERA

STUDIES ON THERMOPHILIC BACTERIA

LETHE E. MORRISON WITH FRED W. TANNER

A new interest in thermophilic bacteria has rapidly developed since the investigations of BARLOW (2), WEINZIRL (35), CHEYNEY (9), DONK (12), and BIGELOW and ESTY (4) have shown their significance in the spoilage of canned foods. The wide distribution of these organisms in nature is shown by the various publications reporting them in water, sewage, snow, canned foods, cotton, hay, manure, soil, etc. Infection of canned foods probably results from the raw materials and water used in the packing process.

History

In a previous paper by MORRISON and TANNER (21) a review of the literature dealing with these microorganisms was presented. Since the experimental work reported in the present paper was concerned principally with the thermal relations and thermal death points, table I was prepared to show these relations for the thermophiles which have been described by various investigators.

Experimentation

SOURCES OF CULTURES.—Cultures 53-87 inclusive, used in this investigation, were isolated from samples of soils, and hog and cow feces. The samples of soils were collected from fields which had received different treatments. All of the specimens of soil examined contained thermophilic bacteria. Cultures 1-52 inclusive were isolated from water. Cultures 88 and 89 were isolated from "Ever Fresh Milk," a commercial bottled milk.

METHODS.—Inoculations into different media were made either from 24-hour agar slant cultures or broth cultures. The Descriptive Chart of the Society of American Bacteriologists was used for recording the salient characteristics, and the index number determined under as uniform conditions as possible. Pathogenicity studies were not carried out with all of the strains. One which was used showed no pathogenic properties when inoculated into guinea pigs. This place in the index number was filled in with zero.

TABLE I

Investigator	Organisms described	Source	Temperatures for growth (C.)	Thermal death points of spores (C.)
Flügge (1894)	Many bacteria, unnamed	Sterilized milk	24°–44° or 27°–54°	Withstood 0.75–5 hours heating at 100°
Rabinowitsch (1895)	Bacillus thermophilus (eight varieties)	Soil, snow, water, milk, excreta, etc.	34°–75°	Survived steam at 100° for 5–6 hours
Karlinski (1895)	Bacillus Illidzensis	Hot springs of Illidze in Bosnia	50°–58°	Withstood flowing steam at 100° for 4 minutes
Kedzoir (1896)	Thermophilic Cladothrix form	River Spree	35°–65°; 35°; opt.	Killed by flowing steam at 100° in 4 minutes
Laxa (1898)	Clostridium gelatinosum	Füllmasse in sugar manufacture	25°–58°	Not killed by exposure to dry heat at 150° for 15 minutes, or moist heat at 100° for 75 minutes
Tsilinsky (1899)	Thermoactinomyces vulgaris	Soil	48°–68°; 35°; opt.	Not killed after 20 minutes at 100° in autoclave; spores of second organism killed in 1 minute at 100°; withstood dry heat for 3 hours at 80°
	Thermomyces lanuginosus	Soil	42°–60°; 54°–55°; opt.	
Sames (1900)	Bacillus I	Earth	56°–70°; opt. 70°–74°; max.	At 750.2 mm. pressure resisted live steam for 3 hours, 10 minutes; spores were formed at 62°
	Bacillus II	Pus from mouse injected with earth and immunized against tetanus	50°–70°; opt. 75°; max.	Resisted steam at 743.7 mm. pressure for 2 hours, 50 minutes; spores were formed at 62°
	Bacillus III	Vaginal mucous during pregnancy	56°–70°; opt. 66°–70°; max.	Resistance of spores formed at 62° at 740.7 mm. pressure = 25 minutes
	Bacillus IV	Raw milk	50°–60°; opt 66°–70°; max.	Resistance of spores formed at 56° to steam at 746.1 mm. pressure = 15 minutes
	Bacillus V	Aqueous litmus solution	56°–62°; opt. 63°–56°; max.	Resistance of spores formed at 62° to steam at 747.3 mm. pressure = 210 seconds; at 37° at 750.6 mm. pressure = 60 seconds
	Bacillus VI	Same as Bacillus II	56°–70°; opt. 66°–70°; max.	Resistance of spores formed at 62° to steam at 746.1 mm. = 13 minutes; at 37° at 745.3 mm. = 60–70 minutes
	Bacillus VII	Air	50°–60°; opt. 66°–70°; max.	Resistance of spores formed at 56° to steam at 749.3 mm. = 100 minutes; at 749.3 mm. = 60–70 minutes
	Bacillus VIII	Earth	50°–60°; opt. 66°–70°; max.	Resistance of spores formed at 56° to steam at 745.1 mm. = 120 minutes; at 37° at 748.9 mm. = 60–75 minutes
Russell and Hastings (1902)	Micrococcus form	Pasteurized milk	20°–25°; opt. 76°; max.	Thermal death limit = 76° for 10 minutes
Tsilinsky (1903)	Bacille no. 7	Infant feces	24°–57°	Resisted heating for 5 minutes at 100° in autoclave
	Bacille no. 8	Infant feces	37°–60° 57°; opt.	Resisted heating for 5 minutes at 100° in autoclave
	Bacille no. 9	Infant feces	42°–45°; opt.	Resisted heating at 100° in autoclave for 5 minutes
	Bacille no. 10	Infant feces	57°; opt. (grew also at 20°)	Resisted heating at 100° in autoclave for 5 minutes
Schardinger (1903)	Group I, no. 1	Foods, etc.	Room temperature 55°–56°; opt	Withstood 2 hours' exposure to boiling water
	Group I, no. 2	Foods, etc.	Room temperature 55°–56°; opt.	Withstood 2 hours' exposure to boiling water
	Group I, no. 5	Foods, etc.	Room temperature 55°–56°; opt.	Killed within 1 hour in boiling water
	Group I, no. 6	Foods, etc.	Room temperature 55°–56°; opt.	Did not withstand exposure of 1 hour in boiling water

Blau (1906)	B. cylindricus	Soil	60°–70°; opt.	Thermal death point 100° for 19–20 hours
	B. robustus	Soil	55°–60°; opt.	Thermal death point 100° for 7.5–8 hours
	B. tostus	Soil	60°–70°; opt.	Thermal death point 100° for 19–20 hours
	B calidus	Soil	60°–65°; opt.	Thermal death point 100° for 7–8 hours
de Kruyff (1910)	Bacterium no. 1	Soil	37°–70°; 60°; opt.	Killed in 5.5–6 hours in boiling water
	no. 2	Soil	45°–73°; 65°; opt.	Killed at 100° in 6.5–7 hours
	no. 3	Soil	38°–60°; 60°; opt.	Killed at 100° in 6.5 hours
	no. 4	Soil	43°–72°; 63°; opt.	Killed at 100° in 8 hours
	no. 5	Soil	39°–67°; 60°; opt.	Killed at 100° in 6.5 hours
	no. 6	Soil	35°–73°; 65°; opt.	Killed at 100° in 4.5 hours
	no. 7	Soil	35°–70°; 55°; opt.	Killed at 100° in 5.5–6 hours
	no. 8	Soil	39°–70°; 58°; opt.	Killed at 100° in 7.5 hours
	no. 9	Soil	38°–67°; 60°; opt.	Killed at 100° in 7 hours
	no. 10	Soil	38°–68°; 60°; opt.	Killed at 100° in 5 hours
Kroulik (1912)	Bacillus I, 1	Soil	60°; opt.	Withstood 2 hours' sterilization with flowing steam
	I, 2	Soil	60°; opt.	Withstood sterilization with flowing steam for 2 hours
	II, 1	Soil	30°–68° 55°–60°; opt.	Resistance greater than that of Bacillus II, 2
	II, 2	Soil	Same as for Bacillus II, 1	Did not withstand action of flowing steam for 2 hours
Negre (1913)	Bacillus 3, 4, 6	Sands of Sahara	70°; max.; 50°; opt.	Resisted heating at 100° for 15–20 minutes
Bergey (1919)	Type 1	Dust, contaminated milk media	50°–60°; min. 75°–80°; max.	Thermal death point at 100°, 400 minutes
	2	Dust, soil	37°; min.; 70°; max.	Thermal death point at 100°, 300 minutes
	3	Dust, soil, horse manure	37°–50°; min. 70°–75°; max.	Thermal death point at 100°, 200 minutes
	4	Same as type 3	50°; min.; 75°–80°; max.	Thermal death point at 100°, 180 minutes
	5 var. *a*	Dust, pig feces, horse manure	37°; min.; 60°–70°; max.	Thermal death point at 100°, 120 minutes
	5 var *b*	Dust, cheese, guinea pig feces	37°; min. 60°; max.	Thermal death point at 100°, 60 minutes
	6	Horse manure	37°; min.; 60°; max.	Thermal death point at 100°, 5 minutes
	7	Same as type 6	37°; min.; 60°; max.	Thermal death point at 100°, 5 minutes
	8 var. *a*	Rabbit's stomach	60°; min.; 70°; max.	Thermal death point at 100°. 120 minutes
	8 var. *b*	Contamination on agar	60°; min.; 70°; max.	Thermal death point at 100°, 120 minutes
	9	Same as type 3	20°–37°; min. 50°–60°; max.	Thermal death point at 100°, 15–60 minutes
Patzschke (1919)	Streptococcus lacticus thermophilus	Milk	....	Thermal death point=75° for 3 minutes; resisted temperature of 85° for 10 seconds
Donk (1920)	B. stearothermophilus	Canned corn	45°–75° 50°; opt.	Not killed by processing at 118° for 75 minutes
Bigelow and Esty (1920)	19 thermophilic micro-organisms	....	....	Time necessary to destroy known suspension of spores in medium of known H-ion concentration decreases as temperature increases; H-ion concentration influences time necessary to destroy known suspension of spores at given temperature; initial concentration of spores influences time necessary to sterilize medium of known H-ion concentration at given temperature
Grieg-Smith (1921)	Rod-shaped bacterium	Fermenting wattle-bark	....	Not killed after exposure to 156°–205° for 2.5 hours
Veillon (1922)	Three anaerobic thermophilic bacteria	Vegetables	20°–58°	Did not develop and killed at about 58°

MEDIA AND TECHNIQUE.—With one or two exceptions, the media used in this study were those recommended by the Committee on the Descriptive Chart (CONN 10). All media were tested for sterility by incubation at 55° C. for 12–24 hours before use. All of the cultures were grown at 55° C. Due to rapidity of growth it was unnecessary to incubate test cultures longer than 4 days, except in the case of milk cultures, which were incubated for 7–10 days. Two per cent agar was used because it was found to be more suitable for work at 55° than the standard agar. The latter dried out very quickly, becoming unsuitable for growth of bacteria. Broth was found to be a useful medium for the propagation of cultures, since evaporation caused less serious changes in the medium.

MICROSCOPIC FEATURES.—The microscopic features were determined from carbol fuchsin and Gram stains. For staining flagella, the recent method described by PLIMMER and PAINE (26) was used with a few slight modifications; it gave very satisfactory results. Agar slant cultures 8–12 hours old were used to furnish the young cells required for flagella staining. Some of the growth was removed and put into tubes containing sterile water which had been held at a temperature of 55° C. These water suspensions were kept at 55° for 30–60 minutes; two or three drops were then placed on slides that had been prepared carefully according to the instructions of PLIMMER and PAINE, and held at 55° for 15–60 minutes. The motility was not disturbed by this treatment, and the flagella were more easily demonstrated by staining. These smears were allowed to dry at 55° over night before fixation and staining.

MISCELLANEOUS BIOCHEMICAL REACTIONS.—As stated, not all of the cultures were used for pathogenicity studies. The one culture which was tested was cultured for 18 hours on fresh rabbit's blood agar slant, from which the growth was removed and suspended in sterile salt solution before injection. Guinea pigs were given injections of this suspension intravenously and intraperitoneally. No pathogenic action was found. BRUINI (6) seems to be the only investigator who has attributed pathogenic action to a thermophilic bacterium.

For the determination of gelatin liquefaction the provisional method of the Committee on Bacteriological Technic of the Society of American Bacteriologists was used. It is designed to distinguish "true liquefiers" (organisms producing ecto-enzymes) from bacteria that produce endo-enzymes that are released from the cell after death and cause liquefaction of the gelatin after incubation of the gelatin after long incubation periods. The cultures were given a preliminary incubation for 24–48 hours in a 1 per cent solution of gelatin at 55° C. After culturing in this gelatin solution, the surfaces of gelatin tubes were inoculated by transferring a drop of the medium. After the incubation period the tubes were cooled to determine whether the gelatin has suffered proteolysis. According to this method all of the cultures except nos. 78, 83, and 84 were found to be gelatin liquefiers. To determine the production of nitrites and gas in nitrate media, both nitrate broth and nitrate agar slants were used.

CARBOHYDRATE REACTIONS.—With certain unimportant modifications, the method of BAKER (1) was used for determining the production of acid and gas for dextrose, lactose, and sucrose. Brom thymol blue was added to these carbohydrate broths before the media were tubed and sterilized. The addition of 15 cc. of 0.04 per cent alcoholic solution of this indicator to a liter of the carbohydrate broth seemed to be about the right concentration of indicator for the detection of acid formation without inhibiting growth. For the determination of diastasic action starch agar plates were used. Dot inoculations were made in the center of the plate instead of the usual streaks.

OTHER CHARACTERISTICS.—Tests for indol were made in nutrient bröth and in Dunham's peptone solution. Both the nitrosoindol and Ehrlich's tests were used. Ehrlich's reagent was prepared according to the method described by NORTON and SAWYER (23). This test gave more satisfactory results when the tubes were heated slightly. All cultures except no. 81 produced indol.

· For the determination of the production of hydrogen sulphide, nutrient broth made with Witte's peptone, over which a strip of lead acetate paper was suspended by means of the cotton plug, was used. The blackening of the paper indicated hydrogen sulphide formation. Streak cultures on "Bacto Lead Acetate Agar" plates were also used. All but cultures nos. 83 and 84 formed hydrogen sulphide. Litmus milk and sterile milk to which brom cresol purple had been added were used to determine the reactions of the thermophiles in milk. These milk cultures were incubated 7–10 days. Most of the cultures grew well in milk; only two (nos. 54 and 59) produced no apparent change in the milk. Cultures nos. 83 and 84 produced alkali with no other apparent change. The majority of the rest of the cultures showed coagulation and peptonization with an alkaline reaction, some of them having shown a slight preliminary acidity. A few cultures showed slight acidity in milk and a few showed distinct acidity with coagulation, but no digestion of the casein.

TABLE II

Class	Index number	Culture numbers	Numbers of cultures in each class
I........	5111–01120–1232	1, 2, 4, 6, 9, 10, 13–20, 24, 26, 28, 31–41, 43–52, 55, 56, 86, 88	41
II........	5211–01120–1232	3, 5, 7, 8, 11, 12, 21–23, 27, 30	11
III........	5211–01120–1233	25	1
IV........	5121–01120–1233	29	1
V........	5111–02120–1232	53, 54, 57, 59–77, 79, 82, 87, 89	26
VI........	5111–02130–2333	58	1
VII........	5111–02220–1232	78	1
VIII........	5111–01130–2333	80	1
IX........	5212–01130–2333	81	1
X........	5111–02230–1222	83	1
XI........	5111–02230–2222	84	1
XII........	5121–02120–1232	85	1

INDEX NUMBERS.—The index numbers determined for these strains are shown in table II, where they are grouped into classes. There seemed to be no correlation between the index number and the source of the strains. The organisms in class I differed from those in class II only in the location of the spore. Those in class I had central spores and those in class II had polar spores. Location of the spores is probably a character of minor importance in classification and analysis of bacterial groups. Consequently, the bacteria in I and II may be regarded as belonging to the same class when spore formation, disregarding the location, is considered. BUSHNELL (8) has called attention to the difficulties arising when only one characteristic is considered, such as location of the spore in the rod. The two classes (I and II) are left separated in this summary, since the separation is called for on the latest Descriptive Chart and because some of the recent reports on anaerobic spore formers indicate this to be a fairly constant characteristic. HALL (15) stated that three distinct groups of anaerobic spore formers could be differentiated on location of the spore: (I) bacteria with central spores which do not swell the rods; (II) subterminal or clostridial spores; and (III) terminal or plectridial spores (*a*, round spores; *b*, elongated spores).

Combining classes I and II, a total of 53 strains is included. This combined class is separated from class V on the basis of relation to combined oxygen. The strains in I and II showed no growth in the closed arm of the fermentation tube in the presence of dextrose. Those in class V showed growth under such conditions. The other classes (III, IV, VI, VII, VIII, IX, X, XI, XII) contain only one strain each, these being separated from the other larger groups on the basis of characteristics of minor importance in systematic studies, such as action on starch, location of flagella, etc. This comparison seems to indicate that the thermophilic bacteria have all of the important characteristics in common.

THERMAL RELATIONS.—Of the 89 cultures discussed in this investigation, 18 were chosen for intensive study of temperature relations and thermal death point determinations. The choice of these 18 cultures was made to include organisms from as many sources as possible. These cultures were grown on agar slants, in gelatin tubes, and on agar plates, at the five different temperatures available for incubation in these laboratories. The agar slants, 5 for each culture, were inoculated by means of a small wire loop from a 24-hour broth culture of the organism to be tested, and these agar slants incubated simultaneously at the five given temperatures. The gelatin was inoculated by placing a loopful of 24-hour gelatin solution culture on the surface of gelatin in tubes. Five tubes were prepared for each organism, and these tubes incubated at the five given temperatures. Large Petri dishes were used for the agar plate cultures, dot inoculations being made in the center of the plate on the surface of the hardened agar. A small wire loop was used to make these inoculations from 24-hour broth cultures of the organisms.

All of these media were incubated at the five given temperatures for 24 hours in order to have a definite period of incubation; a 24-hour incubation period was used because the agar streak cultures and plate cultures seemed to reach their maximum growth within such a period. The comparative amount of growth attained in gelatin and on agar slants at the different temperatures was judged as carefully and as accurately as possible with the naked eye; the diameter in millimeters of the giant colonies produced on the agar plates at these same temperatures was measured.

TABLE III*

GROWTH OF THERMOPHILES ON THREE DIFFERENT MEDIA AT FIVE TEMPERATURES (C.)

Culture number	Agar slant					Gelatin					Agar plate				
	25°–30°	37°	42°–45°	55°	60°	25°–30°	37°	42°–45°	55°	60°	25°–30°	37°	42°–45°	55°	60°
1......	5	4	3	1	2	3	2	1	3	....	4	4	2	1	3
10......	....	3	2	1	....	4	3	2	1	....	5	4	1	2	3
19......	4	3	2	1	3	5	2	1	3	4	5	2	1	3	4
21......	....	4	3	1	2	5	2	1	3	4	5	3	1	2	4
26......	....	4	2	1	3	....	3	2	1	4	4	4	2	1	3
33......	5	4	2	1	3	4	3	1	2	....	5	4	2	1	3
37......	5	4	3	1	2	5	4	3	1	2	4	4	2	1	3
39......	5	4	2	1	3	....	4	1	2	3	4	4	3	2	1
46......	....	4	3	1	2	5	4	2	1	3	4	4	1	2	3
47......	....	4	3	2	1	5	2	1	3	4	5	4	1	2	3
51......	....	4	3	2	1	5	2	1	3	4	4	3	2	1	2
52......	....	4	3	2	1	5	2	1	3	4	4	3	1	1	2
53......	4	3	3	2	1	5	4	3	2	1	4	3	3	1	2
59......	4	3	3	1	2	....	3	1	2	4	4	3	2	1	3
64......	....	4	2	1	3	....	3	2	1	4	5	4	3	1	2
67......	5	3	2	1	4	....	3	2	1	4	4	3	2	1	3
80......	....	....	3	2	1	....	4	3	2	1	....	....	3	1	2
89......	....	4	3	1	2	5	4	3	2	1	....	....	3	2	1

* Figures indicate amount of growth, 1 indicating greatest amount of growth at the stated temperatures, 2 the next best, etc.

The data secured are given in table III.

With a few modifications, the method proposed by BIGELOW and ESTY (4) for the determination of thermal death points of typical thermophilic organisms was followed. Nutrient agar slants were inoculated with pure cultures of the organism to be tested, and grown at 55° for 48 hours. The growth from two agar slants of each culture was brought into suspension by pouring 10 cc. of sterile nutrient broth on to the slants and emulsifying. These suspensions were then transferred to flasks containing 100 cc. of sterile broth and incubated for 7 days at 55° C. At the end of the incubation period, the flasks were placed in a refrigerator for 24–48 hours, when they were heated to 85° for 15 minutes to kill all vegetative forms, cooled immediately, and placed again in the refrigerator to prevent the germination of spores. These were the stock suspensions used for the determination of the thermal death points of the spores. The concentration of spores in the suspensions was determined by plating in different dilutions. The relative ability of the 18 cultures to form spores under these conditions is shown by the counts. The number of spores per cubic centimeter of suspension for the 18 cultures varied from 390 to 73,800,000.

The tubes used for the determinations were hard glass tubes 5 mm. in diameter and 250 mm. in length. They were prepared for use by soaking over night in weak hydrochloric acid solution, rinsing thoroughly with distilled water, draining, wrapping in heavy brown paper in packages of 15 each, and sterilizing. These tubes were inoculated with 1 cc. of the suspensions of spore, sealed off to within 40–50 mm. of the surface of the liquid, and held in the refrigerator until ready to be heated.

The thermal death points to the spores at 100°, 105°, 110°, 115°, and 120° C. were determined by immersing the sealed tubes in an oil bath adjusted to the desired temperature. A DeKhotinsky electric bath containing "Crisco" was used to maintain a constant temperature, and a Wasserman test-tube rack for suspending the sealed tubes in the bath. Before immersing in the oil bath, the temperature was increased one degree in order to compensate for the loss in temperature due to the immersion of the tubes in the oil, and 30 seconds was allowed for the heat to reach the center of the tubes and for the temperature to drop to that at which it was previously adjusted, before recording the time.

A series of tubes was exposed to the desired temperature for definite periods of time, a tube being removed at regular intervals and immediately placed in ice water in order to stop the action of the heat on the spores. When cold, the tubes were placed in the refrigerator and held until the sterility of the medium could be determined. Sterility tests were made by pouring agar plates. In most cases, if the spores survived the heat treatment, growth appeared in 24 hours. The changes, if any, in hydrogen-ion concentration were followed by the use of Clark and Lub's indicators. Table IV summarizes the work on thermal death point determinations.

TABLE IV

TIME REQUIRED TO DESTROY SPORES OF THERMOPHILES AT STATED TEMPERATURES (C.)

Culture number	Spores per cc.	Time required in minutes for destruction at				
		100°	105°	110°	115°	120°
1........	3,580,000	110	45	7	2.50	2.00
10........	390	15	10	3	2.00	1.00
19........	75,000	40	20	5	2.00	1.50
21........	5,560,000	150	60	5	2.00	1.50
26........	8,900,000	80	30	3	2.00	1.50
33........	27,000	60	50	3	2.00	1.25
37........	800,000	80	60	4	2.00	1.50
39........	123,000	190	40	4	2.00	1.75
46........	40,000	90	50	5	2.00	1.00
47........	176,000	120	30	5	2.00	1.75
51........	275,000	70	20	5	2.50	1.50
52........	73,000,000	60	30	7	2.00	1.25
53........	184,000	35	25	7	2.00	1.00
59........	144,000	70	40	7	3.00	1.50
64........	30,000	70	50	5	2.50	1.50
67........	1,644,000	90	60	5	2.00	1.25
80........	500,000	220	70	9	3.00	1.50
89........	11,000	180	60	9	3.00	1.75

Discussion

The thermophilic bacteria seem to constitute a homogeneous group having the more important characteristics in common. That the function of ability to grow at 55° C. or above may be a relative one is suggested by the results reported in this paper and the work of OPRESCU (24), SAMES (30), BLAU (5), and KOCH and HOFFMAN (18), since the conditions seemed to have much influence on growth at the higher temperatures. Table III shows that the organisms grew better at the higher temperatures on agar slants and plates than in gelatin. Some of those which showed no growth at all, or only slight growth, on agar slants after 24 hours incubation at 25°–30° C. were found to grow well in gelatin at this temperature, causing some liquefaction. The time must be considered in connection with the growth of thermophiles at any temperature. Some strains showed no growth on agar slants at room temperature in 24 or 48 hours, but showed slight growth at this temperature after long incubation. KOCH and HOFFMAN stated that their organisms would not grow in artificial media at 25°–28°, but failed to mention the conditions of incubation. It is possible that statements by several investigators that the thermophilic bacteria would not grow at room temperature may have been made without waiting a sufficient period for growth to appear. The three anaerobic thermophilic bacteria isolated by VEILLON (34), however, gave very restricted growth at room temperature even though held for two to three months. RUSSELL and HASTINGS (29) and BIGELOW and ESTY (4) seem to be the only investigators who have described their technique for determining thermal death points. The results obtained in this investigation are at variance in some respects with the results reported by BIGELOW and ESTY on 19 strains of thermophiles. The strains used by the writers seem to be less resistant than those of BIGELOW and ESTY. Several reasons may obtain to explain this. Their strains were isolated from canned foods which had undergone spoilage, and had thus passed through a selective treatment which would give only strains which were resistant to heat. On the other hand, the strains used in this investigation were isolated from nature, with two exceptions, and had not undergone any such treatment. Slightly different P_H concentrations also obtained in the two investigations. BIGELOW and ESTY report a P_H of 6.0–6.3, while a P_H of 7.4–8.0 obtained in this investigation.

While the initial concentration of spores per cubic centimeter has great influence on thermal death point data, the thermophiles, like other bacteria, vary in their degree of resistance to heat. This is shown by the data in table IV. The suspension of no. 10 contained the least number of spores per cubic centimeter; these were killed by a 15-minute exposure to 100° C. Culture 89 required an exposure of 180 minutes for complete destruction. This difference in the time required cannot be explained solely on the variation in resistance, but apparently is influenced also by the number of spores.

A review of the thermophilic bacteria described in the literature indicates that they have a wide temperature range of growth. In order to express this, different investigators have coined different terms to apply to the bacteria in this group, as follows: thermophiles, orthothermophiles, thermotolerants, obligate thermophiles, true thermophiles, facultative thermophiles, and strict thermophiles. Some divisions have been made on the basis of minimum temperature for growth, others on the basis of maximum temperature, and some on the basis of optimum temperature. The best basis for separation, perhaps, is the optimum temperature for growth. This might allow the following grouping of bacteria according to the temperatures at which they grow best.

I. Strict thermophiles.—Optimum temperature above 55° C.
II. Facultative thermophiles.—Optimum temperature 50°–55° C.
III. Thermotolerant bacteria.—Optimum temperature 40°–50° C.
IV. Mesophilic bacteria.—Optimum temperature 25°–40° C.
V. Psychrotolerant bacteria.—Optimum temperature 10°–25° C.
VI. Facultative psychrophiles.—Optimum temperature 0°–10° C. (?).
VII. Strict psychrophiles.—Optimum temperature below 0° C. (?).

Since the growth of thermophilic bacteria at ordinary temperatures seems to involve a time element, it is probable that canned foods containing thermophilic bacteria might spoil after being stored for a long time at ordinary temperatures. Of course, if stored at higher temperatures it is well known that spoilage occurs very promptly.

Several other problems in connection with thermophilic bacteria have been suggested by the present investigation. These will be taken up in the near future.

Summary

1. Eighty-nine strains of bacteria, studied according to the index number as expressed on the descriptive chart of the Society of American Bacteriologists, fell into twelve classes. All of these separations are based upon unessential characteristics of classification. In the light of this fact, the thermophilic group seems to

be a homogeneous one, the members having in common all of the important characteristics used in classification.

2. A tentative separation of bacteria into groups on the basis of reaction to temperature has been presented.

3. The ability to grow at high temperatures seems to be a somewhat relative function, depending on the conditions of incubation. The so-called strict thermophilic bacteria, however, show a much more rapid growth at 55° C. than at lower temperatures. They must be distinguished from thermo-tolerant bacteria.

4. The thermal resistance of eighteen strains of thermophilic bacteria was studied. At the higher temperatures, 115° and 120° C., the thermal death points fell within narrower time limits than those at the lower temperatures, 100°, 105°, and 110° C.

5. The "index number" is a distinct improvement upon the old "group number," although some of the points in the "index number" would be difficult to determine. For instance, the function of pathogenicity could not be determined without a great deal of experimental work.

6. The character of the media influenced the temperatures for the growth of thermophiles.

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[1] There seems to be some confusion in the literature with regard to the spelling of this investigator's name. The two papers published in the *Annals l'Institute Pasteur* bear the name with two different spellings. The names of some of the other investigators are also found with two different spellings. This accounts for a few mistakes in the names of authors in the previous publication by the present authors.

DILUVIAN FLORA OF POLAND

ANIELA KOZLOWSKA

(WITH FIVE FIGURES)

Introduction

Few geological epochs have been so widely discussed, or made the subject of so many diverse theories, as has the Diluvian epoch. Especially in Europe, glacial formations, as well as their accompanying flora and fauna, have been the subject of many varying theories as to climate and general characters of the epoch. Besides being intensely interesting, this subject is also important in connection with the immediate flora of today.

Two fundamental theories by contemporary students stand out conspicuously. The first and oldest, recognized by a small group of scientists, accepts the existence of only one glacial period, with several thawing periods, causing the appearance of a series of moraines, especially in the central part of Europe. The second theory, that of a series of glacial and interglacial periods, recognized by botanists, zoologists, and archeologists of the old as well as the new world, assumes the existence of several glacial periods, followed by a few successive climatic changes of alternate cold and warm spells (5, 19). Although the number of the individual glacial periods for America has been determined (2), there are various opinions as to the number in Europe (7). It is the aim of contemporary students of glaciology, however, to determine chronologically the number and character of glacial phenomena in both continents. In the present paper, the terminology of PENCK and BRUCKNER (19) is accepted as the standard.

Glacial periods in Poland

The first two Alpine glacial periods, Günz and Mündel, did not leave in Europe any definitely ascertained terminal moraines. The third period, Riss, however, has left throughout the whole continent a wall of moraine and many fluvioglacial formations.

In Poland the limit of the so-called "first and largest glacial period" runs through Cracow and Lwow, then turns northward, passing between Wolyn and Podole. This glacier was accompanied by a rich northern tundra, found by ZMUDA (27) and SZAFER (23), near Cracow and in Krystynopol.

Willows and dwarfed birches, such as *Betula nana*, *Salix herbacea*, and *S. myrtilloides*, were then growing on the outskirts of the glacier. This flora did not last long, however, since the glacier retreating from Cracow caused a rapid succession of changes in the vegetation (15). On the treeless tundra there appeared high region trees, such as *Pinus cembra* and *Larix Polonica*. The climate continued to undergo extreme changes. From terrains bordering on Poland, that is, from Germany (24, 25), Silesia (8), Hungary (16, 17), and Russia (22), we have several remains of the forest flora, indicating a warmer climate than that of today. It is the so-called second Interglacial in Europe, corresponding to the Alpine Riss-Würm. The next Alpine glacial period, Würm, is as clearly defined in Poland as the first; it reached the upper courses of the rivers Pilica and Bug. In conjunction with this glacial period there were formed younger loess, covering southern Germany, Poland, and Russia. The flora of this period in Poland is known through a very few remains from the caves in Ojców (13).

Finally, the fifth and last Alpine glacial period corresponds to the Baltic moraine in Poland, which extends through Prussia and Pomerania (fig. 1). The remains of the flora and fauna in these terrains are not sufficiently abundant or characteristic to serve as data from which to determine positively the climatic changes and the contemporaneous changes in the vegetation of Poland. This problem, being of a general nature, still remains unsolved, and presents many puzzling points, in spite of the numerous fossil remains and the extensive research carried on in European countries and in North America. Possibly the following description, containing a list of the Diluvian flora from the region of Czarna River, found in Poland during the year 1921, may throw some light on the problem.

Fossil flora from region of Czarna River[1]

The Czarna River flows at the southern extremities of the Swiętokrzyskie Mountains on the terrain occupied by the largest glacial period (Riss), and not covered by deposits of the second glacial period (Würm), which reached only as far as the northern waterfalls of the Swiętokrzyskie range. The entire shore of this stream is covered by glacial deposits, gravel, sand, and loess. Along its entire course the stream forces its way through these deposits, forming deep ravines, which reveal very interesting sections containing abundant fossil plants.

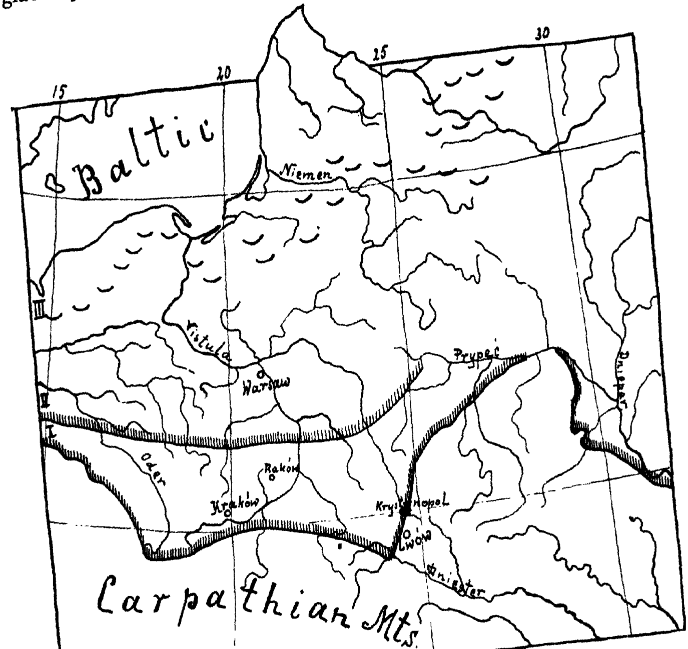

FIG. 1

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[1] A more detailed account of the flora found in this region, as well as a thorough description of all the remains, will appear in a Polish publication in the near future.

Directly on the bluish loam, which covers the bottom of the stream, there appear layers one to two meters thick of fluvio-glacial gravel. Above there lies a homogeneous layer of humus, in parts attaining a thickness of one meter. Upon this there stretch indefinitely described strata of diluvian sands. These formations are finally covered by clay, one to three meters thick, resembling in character loess. It lacks, however, the snails typical of loess, such as *Pupa muscorum*, *Helix hispida*, etc.

The humus, dissected by water, showed excellently preserved remains of a forest. It contained leaves with their veins completely preserved, pine needles, seeds, absolutely undamaged, also many fragments of trees, sometimes with the entire roots protruding from the sands. At a width of one kilometer all along the stream the layer of fossilized forest could be detected always in the same stratigraphic position. A number of specimens from various parts, when analyzed, disclosed a homogeneous character of the forest type. In the individual tests, one species or another prevailed only in quantity. The most abundant species of trees were found to be *Fagus silvatica*, *Abies pectinata*, *Carpinus Betulus*, *Tilia platyphyllos*, *Corylus Avellana*, *Pinus silvestris*, *Acer Platanus*, *A. platanoides*, *Betula verrucosa*, *B. pubescens*, *Ulmus* sp., and the herbs *Stachys silvatica*, *Carex* sp., *Vaccinium uliginosum*, *Viscum album*, and *Cirsium* sp.

It is evident from this list that the fossil forest did not vary much in character from that which now covers the hillsides of the Swiętokrzyskie Mountains. The beech and the fir, the commonest of the fossil species, today also compose the greater part of the forests in these regions. Only *Tilia platyphyllos*, one of the now thriving species of our flora, does not grow wild in the Swiętokrzyskie Mountains. Owing to their climatic requirements, these trees are scarce on the southern boundaries of Poland.

What renders this flora peculiar in character, and arouses special interest, is the discovery, although in only one section of the forest, of the presence of loam with several branches of coniferous trees, varying in their physical structure from all the species of evergreens known to the writer in northern Europe. I have made a careful study of this loam. By maceration in water, there were washed from this loam the following vegetable remains: one-year old branch, several pieces of two-year old roots with many one-year branches, a few pieces of three and four-year old wood, about 20 cm. long with well preserved bark, all belonging to the previously mentioned coniferous trees. Besides, there were one pine needle, with its structure unfortunately disarranged, about a dozen seeds of reed grass, fragments of roots and leaves of grass and reed grass not definitely described, fruits of hazelnut and beech, a few pieces of wood of the fir and elm, single leaves of moss, and very many spherical objects of diluvian age known as *Cenococcum geophillum.* The appearance of all this material was the same as of that lying in the direct vicinity of the forest. All of these remains disclosed in their structure an absolutely young character, and did not show any traces of the action of water. The one and two-year old branches of coniferous trees had falling bits of bark preserved on their exterior, also tiny gnarls on the side branches, and no traces of having been surrounded by water. Moreover, the delicate leaves of moss and reed grass, simultaneously found, apparently present sufficient proof that this material was not subsequently thrust among the forest humus, but that it represents the original composition, and therefore is contemporaneous with the forest described.

Cross-sections of the branches and roots of coniferous trees, obtained by cutting with a razor, and not treated with any chemicals, revealed a well preserved structure of wood and bark, and permitted the exact tracing of the anatomical structure. The three-year old branches showed the following structure: (1) Lack of resin canals in the wood, in the longitudinal and diagonal cross-sections. (2) Lateral walls of the uniseriate ray cells with simple pits. (3) Tracheids of the wood in a radial cross-section reveal a rather changeable structure. In the autumn wood and partly in the summer wood there is but one row of bordered pits, whereas in the spring wood tracheids of slightly larger dimensions disclose bordered pits arranged in two rows. Transitional forms are met with occasionally; in one and the same tracheid, the pits place themselves either singly or doubly. (4) Resin cells of a dark brown hue, scattered along the summer wood. These characteristics establish beyond doubt the relationship of this wood to the Abietineae type, therefore to one or another of the genera *Abies*, *Pseudolarix*, *Cedrus*, or *Tsuga*.

JEFFREY (**11a**) showed what significance the study of diagonal cross-sections of young branches and roots has for the Abietineae type. The appearance and number of resin canals in the individual species are characteristic of this group. Three genera, *Abies*, *Pseudolarix*, and *Cedrus*, have distinct resin canals in the original wood, and therefore in the pith of the one-year old branches; besides they have many resin canals in the bark. The young roots disclose a similar structure; one sap duct in the original wood and many in the texture of the bark. *Tsuga* alone varies fundamentally in this respect, having only one sap canal in the original wood of the root. There is an entire lack of these canals in the bark and the pith of the branches.

The fossil wood examined showed, in diagonal cross-sections of the roots and branches, a structure corresponding exactly with that of *Tsuga* as described by JEFFREY. A thick layer of bark, surrounding the branches and the roots, does not possess resin canals. It is composed of homogeneous dead cells of the bark texture, which cells are divided by sclerenchyma filaments. Similarly, the original wood of the branch does not show any resin canals; they appear only in the pith of the one-year old root. These characters indicate that this wood belongs to *Tsuga*.

Other characteristics of *Tsuga* have been noticed by PENHALLOW (**20**). In his opinion the disposition of the resin cells on the exterior part of the summer wood is extremely characteristic of *Tsuga*. In wood as young as the observed branches the disposition of the resin cells does not appear in such a typical way, but numerous dark cells are aggregated on the exterior of the spring wood. Finally, a character determining the genus with certainty is the disposition of the bordered pits in the tracheid of the wood. In this genus of the type Abietineae, the species *Tsuga canadensis* exclusively has in its spring wood bordered pits disposed in double rows. This character is typical of our fossil wood, and has been verified in all the cross-sections made by the writer. The discovery of the American species, *Tsuga canadensis*, among the mixed wood characterizing central Europe, throws an important light, first on the determination of the age of the fossil flora of Rakow, and second on the floral history of the glacial period.

As I noticed before, the fossil wood in the Czarna River section lies upon a substratum of stones and gravel, and underlies glacial sands and loam. As this stratum is situated not far from the terminal moraine of the first glaciation (Riss), the stones and gravels appearing at the bottom of the streamlet must be remnants of that glaciation. They probably correspond to the lower moraine. The fossil forest with *Tilia platyphyllos* and the *Tsuga canadensis*, showing climatic conditions milder than today, has been able to grow only after the complete retrogression of the glacier near Cracow. This wood, therefore, will correspond to the interglacial period, called in the Alps Riss-Würm. The next glaciation (Würm), stopping in Poland at the beginning of the rivers Pilica and Bug, has covered with sand and loess the rising of Malopolska. The sand and loam with loess character which cover our fossil wood correspond to that second glaciation.

Interglacial flora of Rakow and other discoveries in Europe and America

In all central Europe, and especially in Germany, Denmark, and Switzerland, we know of a fossil flora very abundant and rich in the number of species from the interglacial period. Many times the exact determination of these fossils has presented great difficulties, allowing various interpretations of the same section by the investigators. It can only be stated with certainty that the numerous fossils of the warm diluvial flora in Europe must be scattered between various interglacial periods. The best known of these interglacial periods is the Riss-Würm, which occurred in Europe between the first and second glaciation.

The essential trait of each of these fossil floras is the appearance among the mixed leaf forests of central Europe of some remnant of the flora corresponding to a warmer climate of south Europe. Among such plants that may be considered as typical in the interglacial period are *Buxus sempervirens* and *Ilex aquifolium*, which have been found in Switzerland (26), Germany (25), and France in the north, far from their present area of distribution. Besides this we have a quantity of other species of similar character, known from occasional discoveries. Thus there have been found in France *Acer opulifolium*, *Laurus canariensis*, *Cercis siliquastrum*, *Ficus carica*, *Evonymus latifolius*, *Juglans regia* (6); in Hungary in Ganöcz and Lueski (17, 18) *Cotinus Coggyria*, *Astragalus hamosus;* in Russia near Oka River *Fagus silvatica*, all of which species have been discovered far from their present limits. A similar phenomenon of the advance of the southern species to the

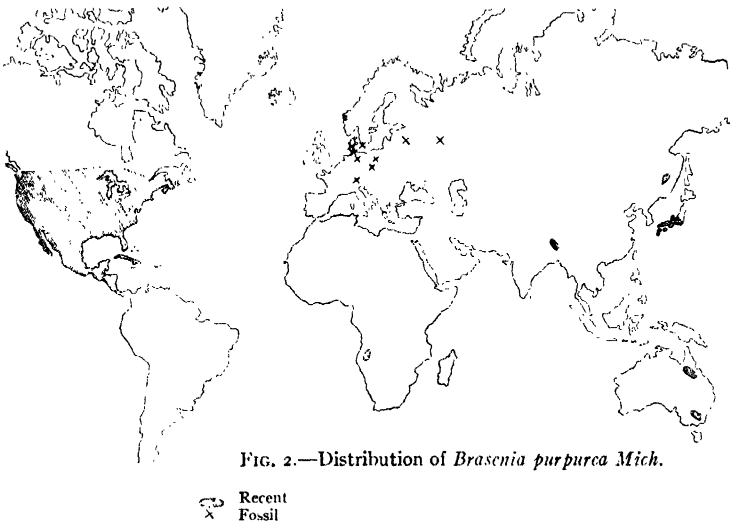
FIG. 2.—Distribution of *Brasenia purpurea Mich.*

north in the interglacial periods has been observed in America. In our fossil flora from Rakow a plant of such a type is *Tilia platyphyllos*, known from many interglacial periods in Europe, which indicates more favorable climatic conditions than the present.

Besides these plant species, there are several plants from the interglacial periods of special interest. These are species which do not exist any more in Europe, but are still found in other parts of the world. *Brassenia purpurea* belongs to these. Fig. 2 gives a view of the distribution of this plant. It originated in North America, where it covers the lakes and rivers from Canada to Mexico; it appears in two places in Africa; it is known as an extremely rare plant in Australia, in the south of Japan, in India, and in Manchuria. In Europe it is no longer in existence, but it has been found in almost all the exposures of the interglacial flora in Germany (14), Denmark (1), Switzerland, and Russia. This widely distributed but scattered species, appearing in several continents, proves that we are dealing with an ancient type, which existed perhaps in the Tertiary period on all of the continents, and today remains only in certain isolated areas. As this species is known in Europe from the Tertiary period, we can assume that in our diluvial epoch it is the last remnant of the abundant Tertiary flora which was almost entirely exterminated during the glaciation. The same may be said of *Nymphaea Lotus*, existing today in Egypt and discovered by PAX (17) in Ganöcz, Hungary.

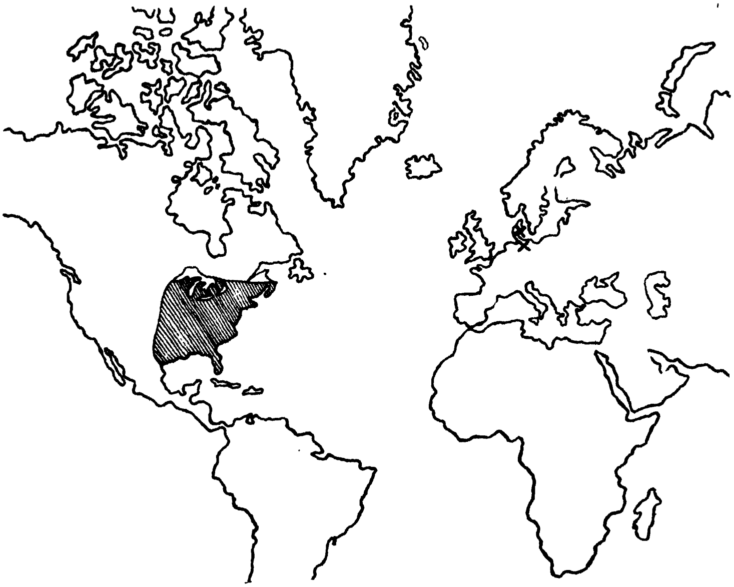

FIG. 3.—Distribution of *Dulichium spathaceum*

▨ Recent
× Fossil

In America, in spite of the comparatively limited studies on the diluvial flora, six species of plants are known from the interglacial period, which are not in existence today, and which are remnants of the Tertiary period (3, 4, 11). Other plants of limited geographic distribution are two species entirely American and found in the European diluvium. One of these is *Dulichium spathaceum*, which today is confined to eastern North America, but which was an element in the flora of Europe (9) in the interglacial period, in Denmark, and in Lauenburg on the Elbe (fig. 3). Moreover, this species is not known in the European Tertiary period. The other species is *Fraxinus americana*, somewhat similar in its distribution to *Dulichium*, limited today to eastern North America. In Europe it has been found in Thuringe by SCHRÖTER (POHLING 21), and is illustrated in fig. 4. *Tsuga canadensis* would represent the third species of similar distribution. It is a northeastern American

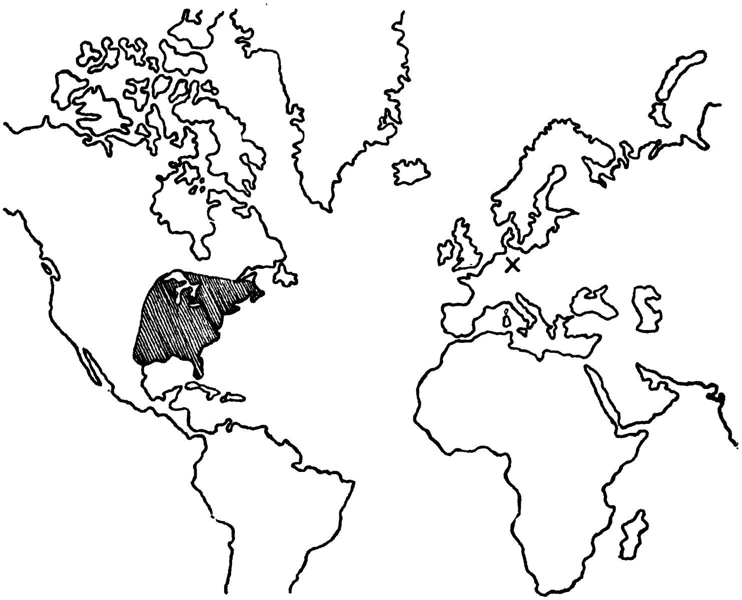

FIG. 4.—Distribution of *Fraxinus Americana*

▨ Recent
× Fossil

species, and does not appear in our existing flora anywhere else (fig. 5).

ENGLER (5) discusses the floral relationship between America, Asia, and Europe on the ground of comparative lists of plant associations of the three continents, and concludes that in the Tertiary there must have been a continental connection between Asia and America, and that migration of the species took place by means of that connection. The lack of identical types in eastern America and western Europe which were not known in Asia (ENGLER quotes only ten types), and the depth of the ocean between Great Britain and Greenland, prove in the opinion of ENGLER a very ancient severing in this region of the two continents. On the other hand, he admits the possibility of a continental connection between America and Europe through Greenland, Spitzbergen, and Nova Zembla. The very rich Miocene flora in Greenland and Spitz-

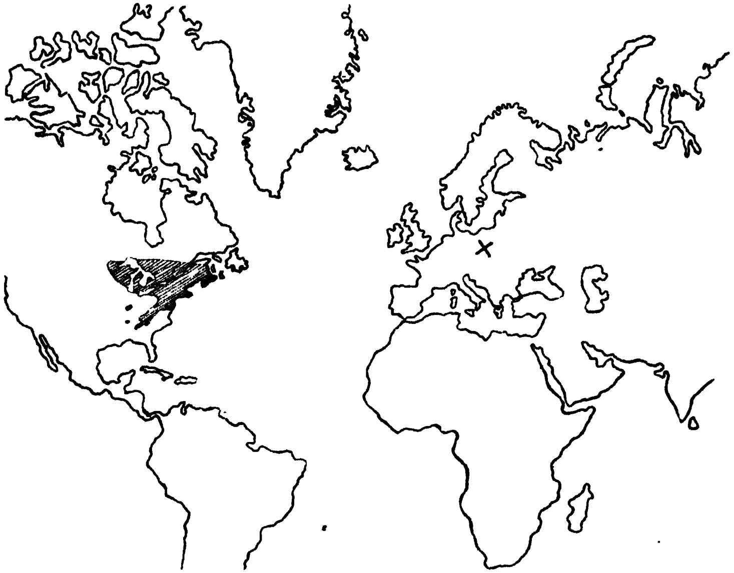

FIG. 5.—Distribution of *Tsuga Canadensis*

▨ Recent
× Fossil

bergen, as described by HEER (10), shows great analogy; the coniferous woods abundantly represented today in North America prevailed there. With the advent of the glacial period all of the species from the boreal region were driven south before the advancing glacier.

In the Miocene flora of Spitzbergen, among the numerous conifers, five species of the *Tsuga* type were discovered, which are not known either in the European or in the American Tertiary period (12). Because of the entire lack of discoveries it is difficult to assume with confidence that this genus did not exist in that period either in Europe or in America. One may assume with a certain degree of probability, however, that the *Tsuga canadensis* discovered near Rakow is a circumpolar species of ENGLER, which in the beginning of the glacial period migrated from Spitzbergen and Greenland to eastern America, and through Nova Zembla has also reached Europe. Whether the connection between America and Europe known only from a few examples can be explained in the same way, remains a problem to be solved by further investigations.

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RELATION OF SEED WEIGHT TO GROWTH AND VARIABILITY OF WHEAT IN WATER CULTURES[1]

SAM F. TRELEASE AND HELEN M. TRELEASE

During the recent development of water culture experimentation, the methods employed in the study of the mineral requirements of plants have been improved rather rapidly. The general method is necessarily so complicated, however, that many of its details still remain to be worked out and perfected. One of the greatest difficulties in the interpretation of the results of water culture studies arises from the high degree of variability of the plants. Variability may obscure the relations between the growth of the plants and the physiological values of the solutions tested. Recently it has become increasingly evident that, to reduce the disturbing influence of plant variability, relatively large numbers of plants must be used in each of the several cultures that are to be compared, and statistical methods must be employed as an aid in the interpretation of the results of such studies (BRENCHLEY 2, 3; STILES 12, 13; STILES and JÖRGENSEN 14; DAVIS 4).

One of the important questions in the general water culture method, therefore, is that of the number of plants needed to secure reliable results. In order to bring the work into conformity with the necessarily limited time and facilities at the disposal of any one experimenter, it is important from a practical point of view to consider methods by which variability may be limited and the number of plants to be grown in each culture may be as small as possible.

To limit the range and degree of variability, investigators have generally used seedlings that appear to be nearly alike in size and general vigor; some workers have used seeds of a pure line, and a few have even employed seeds selected for uniformity in weight (BRENCHLEY 2, 3). It might be anticipated that such precautions would tend to reduce the range of variability in the plants, but further work seems desirable, to determine quantitatively, if possible, just how effective these methods of selection may be. Although it is known that seed weight may have a pronounced influence on plant yields (KIESSELBACH and HELM 7), the importance of this appears not to have been emphasized sufficiently in the literature of the water culture method. It was with the idea of securing further information on this question that the present study was undertaken. This paper reports experiments dealing with the growth and variability of young wheat plants, as related to the original seed weight. It aims to emphasize the importance, in water culture technique, of employing seeds that have been selected for uniformity of original weight.

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[1] Botanical contribution from the Johns Hopkins University, no. 76.

The experiments were carried on at the laboratory of plant physiology of the Johns Hopkins University. It is a pleasure to acknowledge indebtedness to Professor B. E. LIVINGSTON for suggestions in the preparation of this paper.

Experiments and results

The seeds used in this study were from a pure line of Marquis wheat (Marquis, Saskatchewan, no. 70, Selection no. 313), grown near Saskatoon during the summer of 1922. They were supplied by the Department of Field Husbandry of the University of Saskatchewan, through the courtesy of Professor MANLEY CHAMPLIN. In table I is shown the frequency distribution of seed weights, as shown by a random sample of 300 seeds from this lot.

Four groups of seeds were selected on the basis of weight. The first or light group included seeds weighing less than 24.5 mg. The second or medium group included seeds weighing more than 32.5 mg. and less than 36.5 mg. The third or unselected group included seeds taken by "random sampling" from the general stock. The fourth or heavy group included seeds weighing more than 40.5 mg. To expedite the work of securing seeds to represent each of these groups, a sorting by appearance was first made; then the weight group to which each individual seed belonged was determined by means of a balance, and those having the desired weights were saved.

The data for the seeds actually used are presented in table II. The second and third columns present data based on the frequency distribution of weights, derived from table I, the second column showing the ranges of seed weights included in each group, and the third column giving the percentage of seeds falling in each group.

TABLE I

FREQUENCY DISTRIBUTION OF SEED WEIGHTS FOR MARQUIS WHEAT GROWN AT UNIVERSITY OF SASKATCHEWAN DURING SUMMER OF 1922, BASED ON WEIGHINGS MADE OCTOBER 26–30, 1922

WEIGHT CLASS (MG.)	FREQUENCY		WEIGHT CLASS (MG.)	FREQUENCY	
	Actual	Percentage		Actual	Percentage
18	2	0.7	33	22	7.3
19	0	0.0	34	17	5.7
20	1	0.3	35	28	9.3
21	0	0.0	36	25	8.3
22	2	0.7	37	16	5.3
23	4	1.3	38	15	5.0
24	5	1.7	39	19	6.3
25	12	4.0	40	16	5.3
26	3	1.0	41	15	5.0
27	13	4.3	42	8	2.7
28	5	1.7	43	8	2.7
29	14	4.7	44	5	1.7
30	13	4.3	45	3	1.0
31	16	5.3	46	1	0.3
32	12	4.0			

TABLE II

DATA FOR SEEDS OF MARQUIS WHEAT INCLUDED IN EACH WEIGHT GROUP EMPLOYED IN CULTURES

GROUP	DATA BASED ON FREQUENCY DISTRIBUTION OF WEIGHTS		DATA FOR SEEDS CHOSEN FOR EXPERIMENT		
	Inclusive range of weights in group (mg.)	Percentage falling in group	Number chosen	Mean original weight (mg.)	Mean dry weight* (mg.)
Light	18–24	4.7	245	19.3	17.2
Medium	33–36	30.6	281	34.8	31.0
Heavy	41–46	13.4	231	44.5	39.6
Unselected	18–46	100.0	250	33.5	29.8

* Calculation based on determinations of moisture content of similar seeds from same lot.

The third column shows that, if one hundred seeds were taken at random, about five would fall in the light group, about thirty-one in the medium group, and about thirteen in the heavy group. The last three columns of table II give data for each group of seeds as actually employed in the experiment. The fourth column shows the number of seeds chosen to represent each group, the fifth column shows the mean original weight of these seeds, and the sixth column shows the mean dry weight of the seeds (based on determinations of moisture content made with similar seeds from the same lot). Eighty seedlings were selected at random from the original lot obtained from the light seeds, and these were divided into two sets, each set being grown in one of the two culture vessels for that group. Corresponding duplicate sets, each containing forty plants, formed the cultures for the medium, unselected, and heavy groups.

The same general methods were employed in this study as were used by TOTTINGHAM (15), SHIVE (11), TRELEASE (16), and other workers. All cultures were in the same solution, which was one productive of good growth. The total concentration corresponded to about 0.5 atmosphere of osmotic pressure at 25° C. The solution contained the following partial volume-molecular concentrations of its constituent salts: 0.00525 M KH_2PO_4, 0.00189 M $Ca(NO_3)_2$, 0.00336 M $MgSO_4$, and 0.00001 M $FeSO_4$. For the three main salts, these partial concentrations correspond to relative molecular proportions of 50 KH_2PO_4, 18 $Ca(NO_3)_2$, and 32 $MgSO_4$.

The culture vessels were 3-gallon, glazed earthenware "butter" jars. Each jar was filled with solution and covered with a paraffined Portland cement top with eight circular openings. The seedlings were mounted in cork stoppers in the usual way, the stoppers being held in the openings in the cement top. Each stopper bore five seedlings, forty seedlings for each of the two culture vessels in each set. The cultures were placed near the margin of a rotating table, so that all of the plants were exposed to approximately the same fluctuations in environmental conditions throughout the period. The solutions were renewed every five days, thus giving approximately the renewal rate suggested by LIVINGSTON (8), each plant being supplied with an amount of solution corresponding to about 53 cc. per day.

The seedlings were grown from November 4 to December 15, 1922, a period of forty-one days. During the first two days the seeds were germinating on wet blotting paper in a damp chamber, and during the next four days the seedlings developed at the surface of tap water, being supported on a netting germinator. The plants were then transferred to the culture jars, in which they grew for thirty-five days. While the plants were growing in the culture solution, the average daily maximum temperature was 26° C., and the average daily minimum was 18.1° C. During this period the corrected evaporation from a Livingston white spherical porous cup atmometer gave a daily mean of 16.3 cc. At the end of the culture period the tops of the eighty individual plants of each group were harvested separately and dried to an approximately constant weight at 102° C. The top dry weight of each individual plant was then determined. The roots of all the eighty plants in a group were harvested together, and their total dry weight was determined. A summary of the results is given in tables III and IV.

TABLE III

MEAN YIELD OF TOPS, ROOTS, AND WHOLE PLANTS FOR WHEAT SEEDLINGS GROWN FOR FORTY-ONE DAYS (NOVEMBER 4 TO DECEMBER 15, 1922)

GROUP	TOPS		ROOTS		WHOLE PLANTS	
	Actual (mg.)	Relative*	Actual (mg.)	Relative*	Actual (mg.)	Relative*
Light	83.4	1.00	10.5	1.00	93.9	1.00
Medium	126.7	1.52	16.3	1.55	143.0	1.52
Unselected	129.5	1.55	16.4	1.56	145.9	1.55
Heavy	143.0	1.71	17.3	1.65	160.3	1.71

* Each relative value shows weight in terms of the weight for the corresponding light group taken as unity.

Discussion

The data of table III, giving the mean yield of wheat seedlings produced from the seeds of each of the four groups, show that these yields are roughly proportional to original mean seed weights. This statement holds true for tops, roots, and whole plants. It is important to ask whether the observed differences among the mean yield values are significant of true differences between the several plant populations with respect to their final dry yields. To determine whether the difference between any two means is significant, the difference is to be considered of course with respect to its own probable error. Data for this consideration are available for top yields only (table IV), individual weights of roots not having been secured. For mean top yield, the difference between the light and medium groups is 43.3 mg.; this difference amounts to 27.1 times its probable error (43.3÷1.6=27.1). Since the odds are overwhelmingly against the occurrence of a deviation as great as or greater than 27.1 times the probable error, it may be concluded that the plants of the medium group really differ from those of the light group by an increase in top yield of about 43.3 mg. In the medium and unselected groups, it is not likely that the difference between the top yield data for these groups is significant, since the observed difference of 2.8 mg. is only 1.2 times its probable error (2.8÷2.3=1.2). The mean seed weights were nearly the same for these two groups, and the top yields do not differ significantly. The next difference, however, that between the yield for the unselected and that for the heavy group, appears to be surely significant, since the

TABLE IV

TOP YIELD DATA FOR WHEAT SEEDLINGS GROWN FOR FORTY-ONE DAYS (NOVEMBER 4 TO DECEMBER 15, 1922)*

Group	Mean top yield in mg. (M)	Standard deviation in mg. (σ)	Probable error of single observation in mg. (E_S)	Probable error of single observation, as percentage of mean (P_S)	Percentage coefficient of variability (C)
Light	83.4±1.3	17.7	11.9	14.3	21.2±1.2
Medium	126.7±1.0	13.5	9.1	7.2	10.7±0.6
Unselected	129.5±2.1	27.4	18.5	14.3	21.2±1.2
Heavy	143.0±1.5	19.5	13.2	9.2	13.6±0.7

*The statistical calculations were made by means of the following standard formulas: Mean $(M)=\frac{\Sigma w}{n}$, Σw being the sum of the individual top weights, and n the number of variates (80). Standard deviation $(\sigma)=\sqrt{\frac{\Sigma v^2}{n}}$, Σv^2 representing the sum of the squared deviations of each weight from the mean, and n the number of variates (80). Probable error of a single observation $(E_S)=\pm 0.6745\times\sigma$, in which 0.6745 is the usual statistical constant, and σ is the standard deviation. Probable error of the mean $(E_M)=\pm\frac{E_S}{\sqrt{n}}$, E_S being the probable error of a single observation, and n the number of variates (80). Probable error of a single observation, this error expressed as percentage of the mean, $(P_S)=100\times\frac{E_S}{M}$, in which E_S represents the probable error of a single observation, and M the mean. Coefficient of variability $(C)=100\times\frac{\sigma}{M}$, σ representing the standard deviation, and M the mean.

difference of 13.5 mg. is 5.2 times its probable error (13.5÷2.6 = 5.2), the odds being several thousands to one against the occurrence of a deviation equaling or exceeding 5.2 times the probable error.

The evidence is very strong, therefore, that the mean top yields are directly related to original seed weights, the heavier seeds producing higher yields. Moreover, it seems reasonable to conclude that the data for root yields are reliable in indicating a similar general relationship between original seed weight and final yield. The relationship is clearly indicated by the actual data for yields of roots and of whole plants (roots plus tops), and there seems to be no reason for regarding it as less definite for these than for top yields.

The relative values in table III show the degree of influence that original seed weight may exert upon yield at the end of forty-one days of growth. If the mean top yield of the light group is considered as having a relative value of 1.00, then the mean top yield of the medium group has a value of 1.52, that for the unselected group (the original mean seed weight for this group being also medium) has a value of 1.55, and that for the heavy group has a value of 1.71. For root yields the corresponding relative mean values are 1.00 for the light group, 1.55 for the medium group, 1.56 for the unselected group, and 1.65 for the heavy group. For yields of whole plants (top plus roots) the corresponding relative values are the same as for top yields. The close agreement between the relative mean values for top and root yields in each group appears to furnish evidence for the proposition that the mean root yields obtained are probably about as reliable as the mean top yields, in spite of the fact that no statistical evidence for root yields is available here. Furthermore, this agreement indicates, of course, that seed weight may be expected to influence both top and root yields in a similar manner and to a similar extent.

Special attention was not given to the relationship of top length in the very young seedlings to growth or variability of the plants at the end of the culture period. Some evidence in this connection was obtained, however, from the measurements of top length, which were made for all seedlings at the end of six days' growth, when the seedlings were transferred from the netting germinators to the culture solutions. These measurements of top length are shown in table V, together with the mean dry weight values for the seeds. These data at least suggest that top length was directly related to initial seed weight.

The preceding discussion has emphasized the pronounced influence which heavy seeds, as compared with light seeds, may have on the dry weight at the end of a growth period of forty-one days. The absolute values for the final yields are clearly greater with seeds of greater weight. It is interesting to note incidentally that the relative increase of dry material, expressed in terms of initial seed weight, was smaller for the heavier seeds than for the lighter seeds. To show this more clearly, data indicating the growth rates for the culture period are presented in table VI.

TABLE V

MEAN DRY WEIGHT OF SEEDS AND MEAN LENGTH OF TOPS OF SEEDLINGS AFTER SIX DAYS' GROWTH

GROUP	SEEDS		TOPS	
	Mean dry weight (mg.)	Difference (mg.)	Mean length (mm.)	Difference (mm.)
Light	17.2	0	88.3±1.1	0
Unselected	29.8	12.6	101.1±0.7	12.8±1.3
Medium	31.0	1.2	102.8±0.8	1.7±1.1
Heavy	39.6	8.6	107.8±0.6	5.0±1.0

Each of the two indices, the ratio of increase to seed weight and the efficiency index, has particular characteristics that must be borne in mind when it is used as a general measure of the rate at which new material accumulates in the plant. In general, the values of each may be expected to vary with the stage of development of the plant. Without going into these considerations in detail, the data in the last two columns of table VI indicate that the seedlings of the light group increased in weight more rapidly, with respect to the original seed weight, than did those of the medium or those of the unselected groups, which in turn increased relatively more rapidly than did the seedlings of the heavy group. It is apparently safe to conclude that, although greater seed weight resulted in greater final product, the heavier seeds did not yield as great a relative increase, in proportion to their initial weight, as did the lighter seeds.

Since the initial weight of the seeds may have a pronounced effect upon the amount of material produced by the plant in its early stages of growth, it follows, of course, that seeds which are uniform in weight should produce plants which exhibit only a narrow range of variation in dry weight. That plants showing a relatively low degree of variability may be secured by employing seeds that have been selected for uniformity in weight is clearly shown by the present tests. This may be observed from table IV, by comparing the statistical data for the medium group with those for the other groups, particularly with those for the unselected group. It is important to remember that only in the medium group were the seeds chosen in such a way as to secure a low range of variation in weight. In the medium group all of the seeds were selected so that their weight lay within a range of 4 mg. (32.5–36.5 mg.), while the unselected seeds had a range in original weight of about 28 mg. (about 18–46 mg.). Table IV shows that the plants of the medium (selected) group had a coefficient of variability of only 10.7±0.6 per cent, while those of the unselected group had a corresponding coefficient of 21.2±1.2 per cent. The difference

TABLE VI

GROWTH RATE AS INDICATED BY ACTUAL INCREASE IN DRY WEIGHT, RATIO OF INCREASE TO DRY WEIGHT (SIMPLE INTEREST RATE), AND THE EFFICIENCY INDEX OF SEEDS (RATE OF CONTINUOUSLY COMPOUNDED INTEREST)

Group	Mean original dry weight (mg.)	Mean increase of dry material* (mg.)	Ratio of increase to seed weight†	Efficiency index of dry weight production‡
Light	17.2	76.7	4.46	1.70
Medium	31.0	112.0	3.61	1.53
Unselected	29.8	116.1	3.90	1.59
Heavy	39.6	120.7	3.05	1.40

* Final dry weight of whole plant minus dry weight of seed.

† Increase divided by dry weight of seed; "simple interest" rate for the 41-day period.

‡ Natural logarithm of final dry weight of whole plant minus natural logarithm of dry weight of seed; representing rate of "continuously compounded interest" for the 41-day period.

may safely be regarded as significant, for it amounts to 8.1 times its probable error ($10.5 \div 1.3 = 8.1$). Thus selection of seeds for uniformity of original weight rendered the variability of the plants grown from them markedly less than it would have been without such seed selection.

To illustrate the value of seed selection as a procedure in water culture technique, it may be of interest to consider the number of plants that would probably have been needed in each culture in order to obtain a certain desired degree of statistical accuracy, and to compare this number for plants from selected seeds with the corresponding number for plants from unselected seeds. A calculation of the required number of plants may be made by rather simple statistical formulas that are commonly used in connection with chemical analysis of variable samples, such as of fruits and soils (DENNY **5**, HAYNES and JUDD **6**, ROBINSON and LLOYD **10**, WAYNICK **17**, WAYNICK and SHARP **18**).

How many plants would have been required in each of two cultures in different nutrient solutions, the plant populations having the same known degree of variability, in order that there might be odds of 31.36 to 1 that a certain percentage difference between the two culture means is indicative of a significant difference between the nutritional values of the two solutions used? The formula here employed may be written: $N = 2 \times (3.2)^2 \times (P_s)^2 \div D^2$, in which N represents the number of plants required, 3.2 is obtained from a table of odds (PEARL and MINER **9**) and corresponds to odds of 31.36 to 1, P_s is the probable error (expressed as percentage of the mean) of a single plant weight, and D is the percentage difference. This formula was used in making the calculations for table VII, which shows the number of plants required in each of two cultures in order that various percentage differences between the two culture means might be considered significant, with an assurance of 31.36 to 1 odds.

In examining the data of this table, the figures shown in the fourth column may be considered first. For the medium group, that is, for the group with seeds selected for uniformity in seed weight, the number of plants required is shown as eighty, which, it will be remembered, is the number of plants actually used in each group. The item for the unselected group shows that it would require 316 plants from unselected seeds to obtain the degree of accuracy secured with the eighty plants from selected seeds. It will be observed that 316 plants would also be required for the light group, and that an intermediate number, 131, would suffice for the heavy group; but it is to be remembered that neither in the light group nor in the heavy group were the seeds really selected for uniformity, those in the light group being chosen merely to have weights below 24.5 mg., and those in the heavy group to have weights above 40.5 mg.

The last column in table VII, which gives the relative number of plants of each group that would be required for a certain degree of statistical accuracy, shows that 3.95 times as many plants would be needed with the unselected group as would suffice with the medium, or selected, group.

TABLE VII

NUMBER OF PLANTS NEEDED IN EACH OF TWO CULTURES IN ORDER THAT VARIOUS PERCENTAGE DIFFERENCES BETWEEN THE TWO CULTURE MEANS MAY BE INDICATIVE (WITH ODDS OF 31.36 TO 1) OF SIGNIFICANT DIFFERENCE BETWEEN THE TWO MEANS

Group	Percentage difference					Relative number of plants required for each group
	1	3	3.64	5	10	
Light	4188	465	316	168	42	3.95
Medium	1062	118	80	42	11	1.00
Unselected	4188	465	316	168	42	3.95
Heavy	1733	193	131	69	17	1.63

The other columns in table VII show the number of plants for each group that would be needed for various degrees of accuracy. The table thus illustrates how the required number of plants changes with the degree of accuracy desired, this number being inversely proportional to the square of the difference (D). For example, the table shows that if the plants exhibit the variability of the medium seed group, 1062 plants should be needed for a 1 per cent difference to be regarded as significant, 118 plants should be needed for a 3 per cent difference to be significant, forty-two plants should be needed for a 5 per cent difference to be significant, and eleven plants should be needed for a 10 per cent difference to be significant. The data of table VII thus serve to illustrate in an emphatic way the main point brought out by the present study. They show that the employment of seeds selected for uniformity of original weight may enable the experimenter to secure reliable results with a much smaller number of individual plants than would be required if unselected seeds were used.

Conclusions

1. The yields of both tops and roots were found to be directly related to original seed weight; light weight seeds gave low yields, medium weight seeds gave medium yields, and heavy weight seeds gave high yields.

2. From the relation between seed weight and yield, it follows that seeds which are nearly uniform in weight should produce plants which exhibit a relatively narrow range of variation in weight, and selection of seeds for uniformity of original weight rendered the variability of the plants grown from them markedly less than it would have been without such seed selection.

3. Top length in the very young seedlings is directly related to initial seed weight, and therefore selection of young seedlings for uniformity of height may exert a similar, although probably less pronounced, influence in making the variability small.

4. Although the yields from heavier seeds were higher than the yields from lighter seeds, the differences in yields were relatively smaller than the differences in seed weight; greater seed weight did not result in a correspondingly greater yield. Thus, per unit of seed weight, the growth rate was higher in plants from lighter seeds than it was in those from heavier seeds.

5. The value in water culture technique of using seeds that are nearly uniform in original weight is emphasized; such seed selection may make it possible to secure reliable results with a much smaller number of plants in each culture than would be required without selection.

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FURTHER OBSERVATIONS ON NONSYMBIOTIC GERMINATION OF ORCHID SEEDS[1]

LEWIS KNUDSON

(WITH THREE FIGURES)

In an earlier paper (5) on the nonsymbiotic germination of orchid seeds, a method was described for germinating seeds under pure culture conditions, and the successful germination of orchid seeds by the use of certain sugars was noted. The practical value of this method was indicated, and certain aspects of the problem respecting the relation of the fungus to germination were considered. It was suggested that germination is dependent upon a supply of organic substances. If the germinating orchid seed is dependent on an available supply of organic matter, then the method should be applicable to seeds of orchids other than those used in the previous experiments. The seeds successfully germinated by the use of sugar were those of *Cattleya*, *Laelia*, and *Epidendron*. No other seeds were available at that time. RAMSBOTTOM (7), reviewing this work of the writer, states that while the method may be useful in the germination of *Cattleya* and related forms, it will not prove successful for the seeds of *Odontoglossum* and related genera. He states, furthermore, that there is not much difficulty in germinating seeds of *Cattleya*, but the fact remains that under pure culture conditions *Cattleya* has not been germinated as yet in the absence of organic matter. Seeds of *Cattleya* and probably seeds of most orchids are unusual in that germination does not occur except when an outside source of carbon is available. In order to discover whether the method is applicable to other seeds, experiments were made with seeds of various genera, and that germination is possible is shown in the experiments here described. Certain other aspects of the problem are also considered.

The methods used were essentially those previously described (5). Solution B or Pfeffer's solution was used throughout, although attention should be given to the hydrogen-ion concentration for best results. Work on this will be reported subsequently by Mr. R. S. NANZ, who is at present studying the subject. The seeds were sterilized by the use of calcium hypochlorite and the tubes and flasks were kept in the greenhouse.

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[1] Since the preparation of this manuscript germination of seeds of *Odontoglossum* and of *Cypripedium* has been obtained.

CYMBIDIUM.—Seeds of a *Cymbidium* hybrid were obtained in May 1922, and twenty tubes were planted on May 8. Ten of the tubes contained solution B+2 per cent sucrose, and ten were made up with solution B alone. In all cases, 1.75 per cent agar was used. On the sugar media growth was rapid, and the mode of germination similar to that described by BERNARD (1). At the end of three months the initial leaf was formed. On the mineral nutrient solution alone, enlargement of the embryo occurred and chlorophyll was produced, but at the end of eleven months no leaf was formed. The germinated seedlings were transplanted September 1 to liter flasks of Erlenmeyer form, each of which contained 300 cc. of solution B+2 per cent sucrose. Growth of these seedlings continued rapidly, and despite the fact that over 100 seedlings were planted in each flask, at the end of eleven months the seedlings averaged 5 cm. in length, having three or four leaves and well developed roots (fig. 1).

ODONTOGLOSSUM.—On the same date a similar experiment was started with seeds of *Odontoglossum Rossii*×*Odontioda* hybrid. These and the seeds of *Cymbidium* were obtained from Mr. A. C. BURRAGE, of Boston, Massachusetts. The results were the same as with *Cymbidium*, except that after transplanting, the seedlings apparently went into a resting condition, growth being resumed near the middle of January. At present the seedlings average 2 cm. in height, with roots developing.

PHALAENOPSIS SCHILLERIANA.—On November 10, 1921, seeds were sown in tubes containing solution B+1 per cent glucose or sucrose. These germinated, and by the end of four months the initial leaf was appearing. Unfortunately, they were transplanted to small flasks in which the agar soon dried up. These seedlings were transplanted again in December 1922. Growth again took place, so that one and two leaves are now formed, and roots four or five mm. in length are now present. Undoubtedly more satisfactory and rapid growth would have been obtained had proper attention been given to the hydrogen-ion concentration.

OPHRYS.—Seeds of four species of *Ophrys* (*O. fusca*, *O. speculum*, *O. apifera*, and *O. fragrans*) were obtained from Spain, and planted on December 26, 1922. On April 1, 1923, the seeds were germinated. The protocorm at this time was a large spherule about 1.5 mm. in diameter, with many radiating absorbing hairs, some of them 1 cm. long. The initial leaf was just appearing. The protocorm was colorless, while the initial leaf was a deep green.

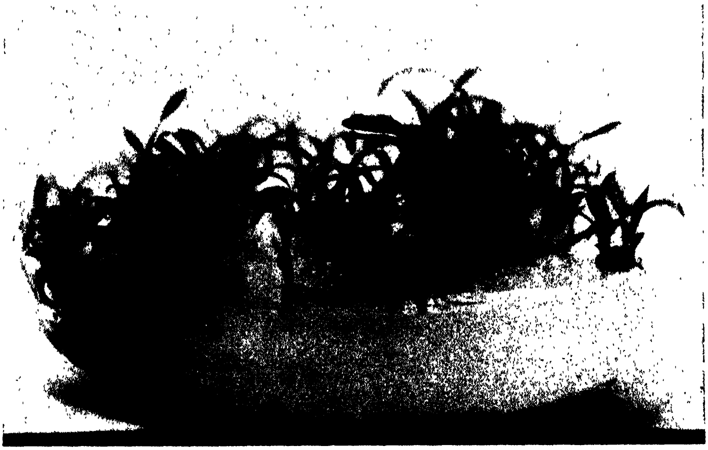

FIG. 1.—Seedlings of *Cymbidium*; 4/5 natural size

DENDROBIUM.—Various experiments have been made with seeds of *Dendrobium nobilis*. No difficulty has been noted in germinating these seeds. After germination in tubes the seedlings were transplanted to flasks, and in from six to seven months the seedlings had four and five leaves, and a small pseudo bulb was formed.

Thus far I have failed only with the seeds of two genera, and I am not certain that the method is at fault, but suspect that the seeds were not viable. Mr. SANDERS of Belgium brought with him from that country a pod of *Odontoglossum* which he gave to me after several weeks of traveling. The pod when received was blackish green, but firm. The seed when examined microscopically appeared immature, although I am not certain that this was the actual case, not being very familiar with seeds of this genus. This seed failed to germinate. In another experiment, seeds of *Cypripedium venustum* were planted, and these also failed to germinate. The age of the seeds was not known, so that failure may have been due to loss in viability.

To sum up this phase of the work, it may be stated that germination has been noted for seeds of *Cattleya*, *Laelia*, *Epidendron*, *Cymbidium*, *Phalaenopsis*, *Dendrobium*, *Ophrys*, and a hybrid of *Odontoglossum*×*Odontioda*. In all cases the germination has been practically 100 per cent.

VIABILITY OF ORCHID SEEDS.—Through the courtesy of LAGER and HURRELL of Summit, New Jersey, seeds of *Cattleya* and related forms and hybrids were obtained for experiments on viability. These were planted on November 6, 1922, and observations recorded on March 1, 1923. Previous to being received, these seeds were kept in a rather warm dry office in paper packages. Five lots of seeds one year old or less germinated readily. Two lots of seeds about fifteen months old did not germinate. Some of the more interesting observations were made with old seeds. Thus seeds of *Cattleya Trianae* which had been stored for three years showed 40 per cent germination. Seeds of *Cattleya Dowiana*×*C. Faleia* which had been stored four years germinated to the extent of 1 per cent. This percentage of germination was obtained with other seeds of this age. Various other lots of seeds three years of age showed 1–10 per cent germination. With seeds of *Cattleya* there is apparently some variability in the duration of viability. No difficulty has been experienced in germinating fresh seeds, but with seeds a year or more old the results are uncertain. It would seem from this that seeds should be planted as soon as possible after collection.

NECESSITY OF ORGANIC MATTER FOR GERMINATION.—In certain experiments, BERNARD found that germination occurred when he used a concentrated solution of salep. This would contain sugars and other organic substances. He ascribed the beneficial effect of the increased concentration to a physical chemical effect, and compared the action with that of high concentrations in changing the form of algae. Recently, RAMSBOTTOM has implied that the effect of the fungus or of high concentration of salep is comparable with the activating influence of various salts and chemical reagents on inducing the parthenogenetic development of certain eggs. That this is not the true explanation in the case of sugars is evident from the various experiments. Whenever the seeds are sown on media containing the appropriate sugar, that sugar is absorbed and stored as starch in the embryo, but not all sugars are utilized. If lactose is used at the same molecular concentration as glucose, fructose, or sucrose, the seed behaves as though no sugar were present. Lactose is not generally utilized by green plants as a food.

That the sugar is used as a food is suggested again by the following experiment. On January 30, 1922, seeds of a *Cattleya* hybrid were sown in tubes on solution B+2 per cent glucose. These cultures were kept in a dark incubator with the temperature maintained at 25° C. On May 3, the seedlings were well advanced and bearing one or two leaves (fig. 2). The seedlings were very much etiolated, but when transplanted to flasks they quickly became green, and are now making a normal growth. The first observations were made on these seedlings on April 8, 1922. At that time the embryos were gorged with starch, and averaged close to 0.5 mm. in width. The same results reported for glucose were also obtained when mannose was used. Without sugar no germination occurred. BURGEFF (4) attempted to germinate seeds of *Cattleya* in the dark, supplying sucrose in the nutrient medium, but he reported that germination was not possible under these conditions. The concentration of sugar was too low and the time of incubation probably not long enough.

In all of these experiments attempts were made to germinate the seeds on nutrient media containing only the essential salts, but lacking in sugar. In all cases germination failed to occur. With seeds of *Cattleya*, no germination was noted even after two years on such media, despite the fact that the embryos were transplanted several times because of the tendency of the media to evaporate. The embryos became green after the first week or so, and gradually increased in size, so that at the end of three months they were 0.2–0.3 mm. in width, and at the end of a year some were found to be 0.5 mm. Beyond this, no further development was noted.

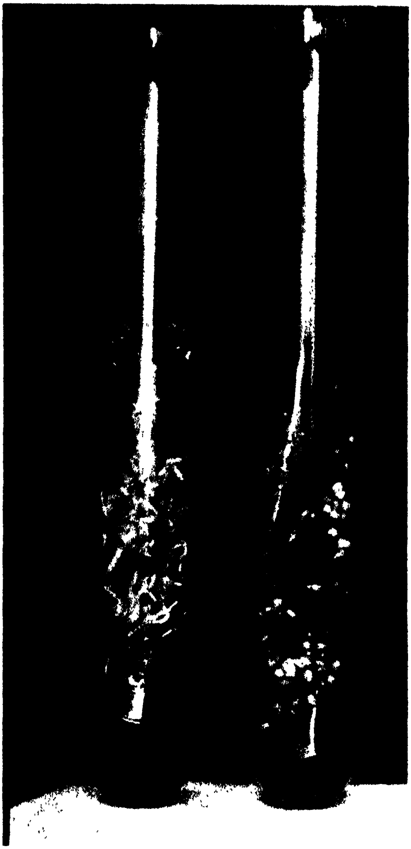

FIG. 2.—Seedlings of *Cattleya* germinated in dark; smaller seedlings on 4 per cent and larger ones on 2 per cent glucose; 4/5 natural size.

What is the cause of this failure to germinate? Chlorophyll is apparently present; water is readily absorbed; delicate absorbing hairs may be formed; all of which indicate complete permeability to salts and gases. It appears likely that the chlorophyll does not function because of the lack of some internal factor which is essential for the photosynthetic process. This would be in line with the recent observations made by BRIGGS (2, 3) and others that the initiation of photosynthetic activity in young leaves is dependent on some internal factor. The photosynthetic activity lags behind chlorophyll development. The fact as reported by BRIGGS that the cotyledons of *Helianthus* after chlorophyll development do not show this delay in the photosynthetic process suggests that food materials are involved. The facts that the orchid seed has practically no reserve food, and that if germination proceeds to a certain stage the embryo becomes independent of any outside food, make the preceding hypothesis more plausible.

It is made apparent from a number of experiments that once germination has proceeded to a certain point, the seedlings become independent of sugar supplied by the medium and independent of any fungus. Seeds of *Cattleya* were sown on solution B+2 per cent sugar in the usual way. When the leaf point was just appearing, that is, when the embryos were about 0.7–0.8 mm. in width, they were transplanted to flasks containing solution B or Pfeffer's solution. No sugar was supplied. While slower than in those cultures with sugar, growth was continuous, and well developed plants were obtained (fig. 3). These results have been noted not only in these two cultures, but in many other cultures of like character.

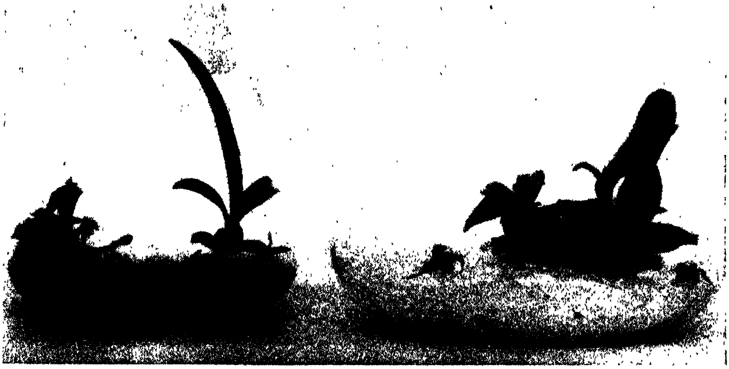

FIG. 3.—Two-year old *Cattleya* and *Laelia Cattleya* seedlings on full nutrient solutions without sugar; 2/5 natural size.

SUMMARY.—These experiments lend further support to the hypothesis that the germination of orchid seeds is dependent on an outside source of organic matter. The effect of sugar on germination is that of a food, but when germination has proceeded to the stage when the leaf is just appearing, then subsequent growth may occur in the absence of any available organic matter in the nutrient medium. This leads to the idea that while the embryos possess chlorophyll, the photosynthetic process does not occur, probably owing to the lack of some internal factor. While these experiments do not disprove the necessity of the fungus for germination under certain conditions, they do emphasize again the nutritional aspects of the problem. At the present time experiments are in progress on the so-called symbiotic germination, and the results thus far obtained lend support to the ideas expressed in my previous paper in respect to the function of the fungus.

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EFFECT OF TEMPERATURE AND LIGHT ON CATALASE CONTENT OF SPIROGYRA

W. E. Burge and E. L. Burge

(WITH TWO FIGURES)

As a result of the work of Lavoisier (6) on animals, and of Priestly (7), Ingen-Housz (5), Senebier (8), and De Saussure (4) on plants, it is now known that both animals and plants in their respiratory processes take up oxygen from the air and give off carbon dioxide. There are many ways of increasing, as well as decreasing this oxygen absorption and carbon dioxide elimination. Exercise, food, cold weather, cold baths are some of the ways by which the gaseous exchange in animals may be increased, while it is decreased during rest, starvation, and anesthesia. The respiratory metabolism in plants may also be increased or decreased in various ways, one of the most effective being by varying the temperature. Light produces only a slight increase in the respiratory metabolism of plants, although it is essential for photosynthesis (2).

During the past several years we have found that whatever increases the respiratory metabolism in animals, whether it be food, exercise, cold baths, or cold weather, invariably produces an increase in catalase, an enzyme possessing the property of liberating oxygen from hydrogen peroxide; and whatever decreases the respiratory metabolism, as rest, starvation, anesthetics, decreases this enzyme. The object of the present investigation was to determine whether an increase or decrease in oxidation in a plant is accompanied by a corresponding increase or decrease in catalase similar to that found to be the case in animals. It should be mentioned in this connection that evidence bearing on this point has already been furnished by Appleman (1) and Crocker and Harrington (3). Appleman found in the greening and sprouting of potatoes an increase in catalase corresponding with the increase in respiration. Crocker and Harrington found a similar relation between catalase and respiration in the germination of seeds.

The plant used in this investigation was *Spirogyra*, and the respiratory metabolism was decreased by lowering the temperature and increased by raising it. The effect of light was also studied. We were fortunate in having an almost inexhaustible supply of a practically pure culture of *Spirogyra porticalis*[1] in a nearby lake. The lake was frozen almost continuously during the months of January and February, when most of the experiments reported in this paper were carried out.

The catalase determinations were made as follows. The desired amount of *Spirogyra* was placed in a cloth and the excess water removed by squeezing. This material was then ground through a small hashing machine twice, and 1.25 gm. of it added to 15 cc. of neutral hydrogen peroxide at 18° C. in a bottle. The amount of oxygen liberated in twenty minutes was taken as measure of the catalase content. The bottles were shaken at a slow rate in a shaking machine during the determinations.

On several successive days the ice on the lake was broken, *Spirogyra* collected, and catalase determinations made. It was found that 1.25 gm. of the macerated *Spirogyra* liberated on the average 27 cc. of oxygen from neutral hydrogen peroxide in twenty minutes, the greatest amount of oxygen liberated being 29 cc. and least 25 cc. A quantity of *Spirogyra* was removed in the ice-cold water to the warm laboratory (22° C.) and catalase determinations made immediately, after four, and after twelve days. The material which was used immediately liberated 29 cc. of oxygen from hydrogen peroxide in twenty minutes; that kept in the warm laboratory for four days, 52 cc., and that kept twelve days, 62 cc. It may be seen that the removal of *Spirogyra* from the cold water of the lake where it had been during the winter to the warm laboratory resulted in more than doubling the catalase content in fourteen days. This is in keeping with the fact that the respiratory metabolism of plants is increased by raising the temperature.

The object of the following experiments was to determine the effect of different temperatures and of artificial light on the catalase content of *Spirogyra*. A large quantity of *Spirogyra* was collected and brought to the laboratory in ice-cold water. It was found that

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[1] We are indebted to Dr. STELLA HAGUE for identifying this species of *Spirogyra*.

1.25 gm. of this material liberated 31 cc. of oxygen from hydrogen peroxide in twenty minutes. The large batch of *Spirogyra* was divided into six portions, each of which was placed in 10 l. of lake water in a container 40 cm. in diameter. Three of the portions were placed in the dark at 0°, 18°, and 30° C., while the remaining three were kept at the same temperatures and each exposed to a 200 watt light with a frosted bulb at a distance of 1 m. Catalase determinations were made at certain intervals. The results are given in fig. 1.

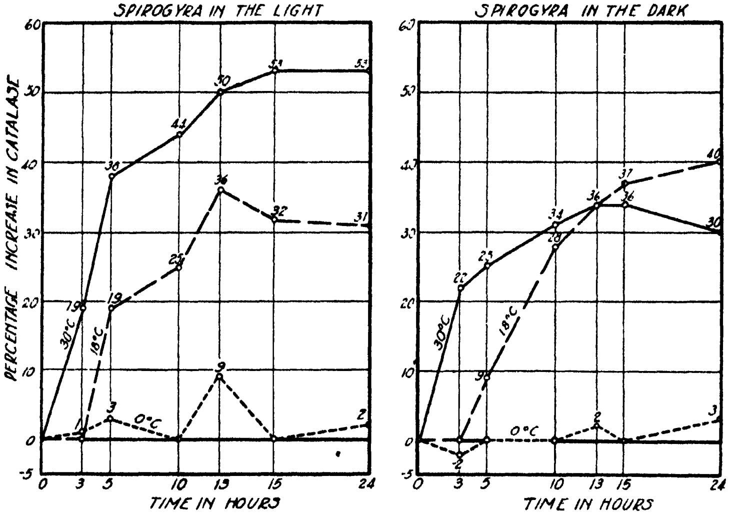

FIG. 1.—Curves showing that rise in temperature increases catalase of *Spirogyra* in light as well as in dark, and that increase is greater in light than dark.

There was no change in the catalase content of the *Spirogyra* kept either in the light or in the dark at 0°, while there was an increase in that kept at 18° and 30° C., both in the light and in the dark. The increase in catalase of the *Spirogyra* kept in the light at 30° was greater than that kept in the dark at the same temperature. This is in keeping with the fact that light, independent of the heat effect, produces an increase in the respiratory metabolism of plants. Two other similar experiments were carried out with essentially the same results.

In fig. 2 is shown the effect on the catalase of keeping *Spirogyra* under ordinary conditions of day and night at 18° and 0° C. The *Spirogyra* that was kept at 18° was placed in the laboratory before a window where the sun could shine on it most of the day, while that kept at 0° was on the roof. No change was produced in the catalase content of the *Spirogyra* kept at 0°, while there was an increase in that at 18° C. After seventy-six hours, a part of the material that was being kept in the laboratory at 18° was lowered to a temperature of 0° C., and the effect was to decrease the catalase greatly.

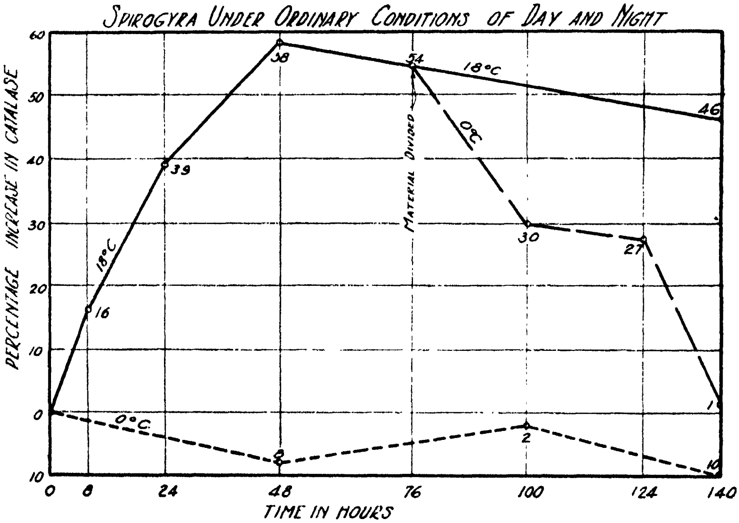

FIG. 2.—Curves showing that rise in temperature increases catalase of *Spirogyra* and a fall decreases it under ordinary conditions of day and night.

Summary

1. A fall in temperature produces a decrease in the catalase of *Spirogyra*, and a rise in temperature an increase, in keeping with the fact that a fall in temperature decreases and a rise in temperature increases the respiratory metabolism of plants.

2. Light also produces an increase in the catalase of *Spirogyra*, less extensive, however, than that brought about by a rise in temperature, in keeping with the fact that light is less effective than a rise in temperature in increasing the metabolism of plants.

3. Whatever increases or decreases the respiratory metabolism in animals produces a corresponding increase or decrease in catalase. From the work here reported and the work of others on the subject, it would seem that it is probable that this same relationship exists between catalase and the respiratory metabolism in plants. Hence the suggestion is made that catalase may be the enzyme in both plants and animals which is principally responsible for the respiratory metabolism.

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DEVELOPMENT OF SEED OF LINARIA VULGARIS

MELVILLE T. COOK

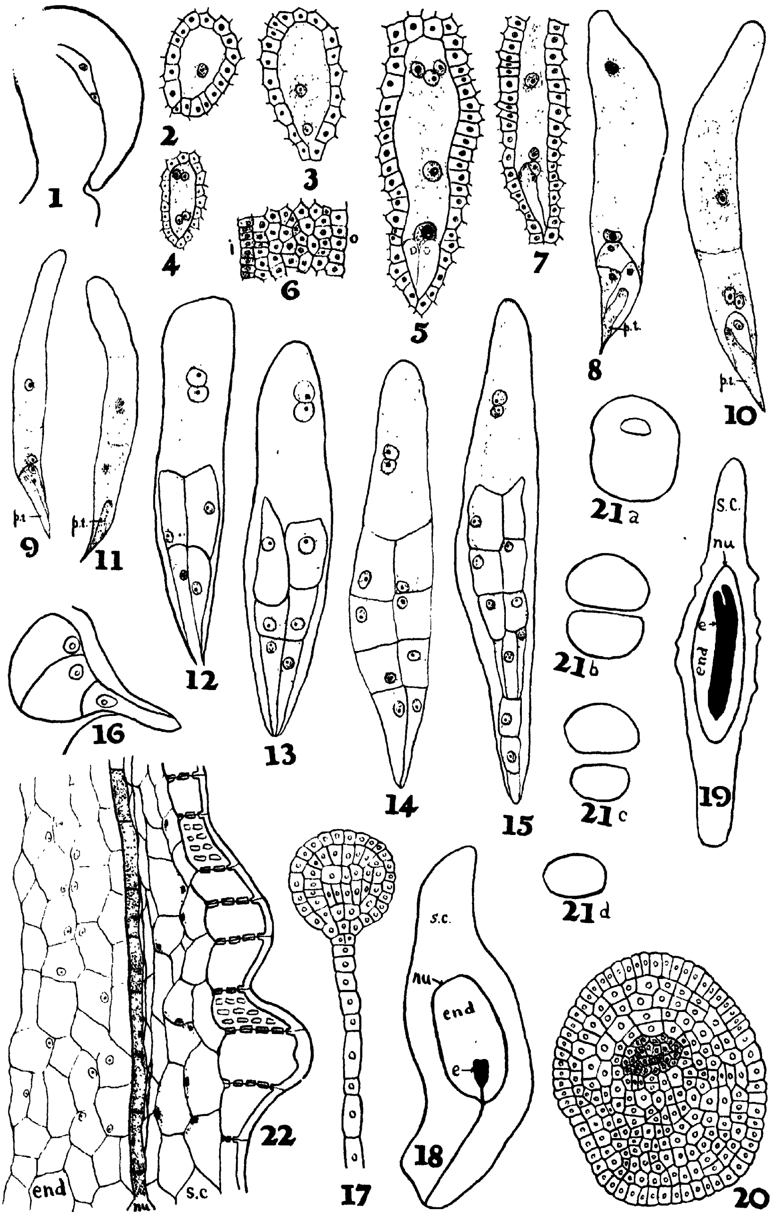
(WITH PLATE XVI)

Linaria vulgaris is an introduced plant belonging to the Scrophulariaceae. It bears a large number of very small seeds, but probably reproduces most generally by means of vegetative organs. The embryo sac is located deep within the ovule, but the pathway of the pollen tube is well defined (figs. 1, 18). The embryo sac develops in the normal manner (figs. 2–5), and is bounded by well-defined, more or less cubical cells, which are richer in protoplasm than the other cells of the nucleus (figs. 2–7). The synergids and antipodals disintegrate very quickly. The polar nuclei unite in the normal manner, but triple fusion was not observed.

The pollen tube passes through a very small canal, enlarges, and becomes very prominent after entering the embryo sac (figs. 1, 8–11). Fertilization was not observed, but probably occurs in the normal manner. The proembryo divides by two transverse walls, the two upper cells enlarging and developing into a spherical embryo, and the lower cell developing into a long suspensor consisting of a single row of cells (figs. 16, 17). This spherical embryo gives rise to the cotyledons and other structures (fig. 17), and at this period bears a striking resemblance to the corresponding stage of the embryo of *Capsella bursa-pastoris*. The development of the cotyledons is unequal, one starting somewhat in advance of the other and developing into a slightly larger organ (figs. 19–21). A cross-section of the embryo just below the cotyledons at this stage shows the fibrovascular bundle of one developing a little in advance of the other (fig. 20), and successive sections farther up show that one cotyledon is always slightly larger than the other (fig. 21*a*–*d*).

The endosperm is much more interesting, and may develop although the embryo does not. In fact, the majority of seeds are without embryos. In some cases all the seeds in an ovary may be without embryos. The development of the endosperm, however, is the same regardless of the presence or absence of the embryo. In some cases the pollen tube enters a sac in which no embryo is formed (fig. 11), but in most cases it is doubtful whether the ovule is penetrated by the pollen tube. The endosperm is of the cellular type, and the walls are well defined. The first division is transverse (figs. 9–11). After this, the development of the endosperm is from the cell next to the micropyle, which undergoes repeated transverse and longitudinal divisions (figs. 10, 12–15). The cells of the endosperm at this stage tend to shrink in most cases from the action of the killing fluid, and give very much the appearance of an embryo in those ovules in which there is no embryo. In fact, the resemblance is so striking that the writer was deceived for some time. The nucleus in the antipodal end of the sac divides, but no wall is formed. The two nuclei lying side by side have very much the appearance of two polar nuclei, and help in the deception (figs. 12–15). The writer was unable to determine their final fate, but it is doubtful whether they serve any further function. A division of the sac into two chambers and the development of the endosperm in the micropylar end of the sac have been described for many species among both monocotyledonous and dicotyledonous plants, including the Scrophulariaceae. The behavior of the endosperm nucleus in the antipodal end of the sac varies in different species; in some there is little or no change, in others there is an enlargement, in others a division into two or three nuclei, and in others a disintegration. The endosperm of *L. vulgaris* finally develops into a compact mass of small cells with thin but well defined walls. These cells are rich in protoplasm until or just before the starting of the cotyledons (fig. 18), when the protoplasm and nuclei begin to disappear and starch is formed in great abundance. The nucellus disappears very rapidly, and at the time of the beginning of the cotyledons it is reduced to a single layer of cells which persists in the mature seed (figs. 18, 19, 22).

The mature seed consists of a very long embryo with short unequal cotyledons (fig. 19), imbedded in a mass of endosperm consisting of large cells filled with starch (figs. 19, 22), surrounded by a single layer of nucellar cells which are rich in protoplasm; all of which is surrounded by a seed coat. This seed coat consists of large thin-walled cells containing practically no food or protoplasm, and an outer layer of large thick-walled pitted cells.



COOK on LINARIA

This work was done at the New York Botanical Garden while the writer was on leave of absence from Rutgers College.

RUTGERS COLLEGE
NEW BRUNSWICK, N.J.

EXPLANATION OF PLATE XVI

FIG. 1.—Ovule, showing embryo sac and pollen tube canal through nucellus.

FIG. 2.—One-celled embryo sac.

FIG. 3.—Two-celled embryo sac.

FIG. 4.—Four-celled embryo sac.

FIG. 5.—Eight-celled embryo sac.

FIG. 6.—Nucellus at time of completion of embryo sac (*i*, inner wall; *o*, outer wall).

FIG. 7.—Embryo sac just before fertilization; no union of polar nuclei.

FIG. 8.—Embryo sac just after fertilization; endosperm nucleus has undergone first division; also showing pollen tube (*pt*).

FIG. 9.—Embryo sac with one-celled embryo, pollen tube, first division of endosperm nucleus and wall.

FIG. 10—Same as fig. 9, but also showing division of endosperm nucleus next to micropyle, but with second wall as yet unformed.

FIG. 11.—First division of endosperm nucleus; no embryo; pollen tube present.

FIG. 12.—Endosperm but no embryo; note two nuclei formed from division of endosperm nucleus in antipodal end of embryo sac.

FIG. 13.—Same as fig. 12 but slightly older.

FIG. 14.—Same as fig. 13 but slightly older.

FIG. 15.—Same as fig. 14 but slightly older.

FIG. 16.—Three-celled embryo.

FIG. 17.—Spherical embryo with long suspensor.

FIG. 18.—Diagram of seed, showing young embryo (*e*), endosperm (*end*), nucellus (*nu*), and seed coats (*sc*).

FIG. 19.—Same as fig. 18, but showing older embryo.

FIG. 20.—Cross-section of embryo just below cotyledons; shaded part indicates fibrovascular bundle in older cotyledon.

FIG. 21*a*, *b*, *c*, *d*.—Four successive cross-sections of embryo as in fig. 20; *a*, same as fig. 20; others in cotyledon region showing differences in two cotyledons.

FIG. 22.—Section of fig. 20, showing endospern, nucellus, and seed coat.

BRIEFER ARTICLES

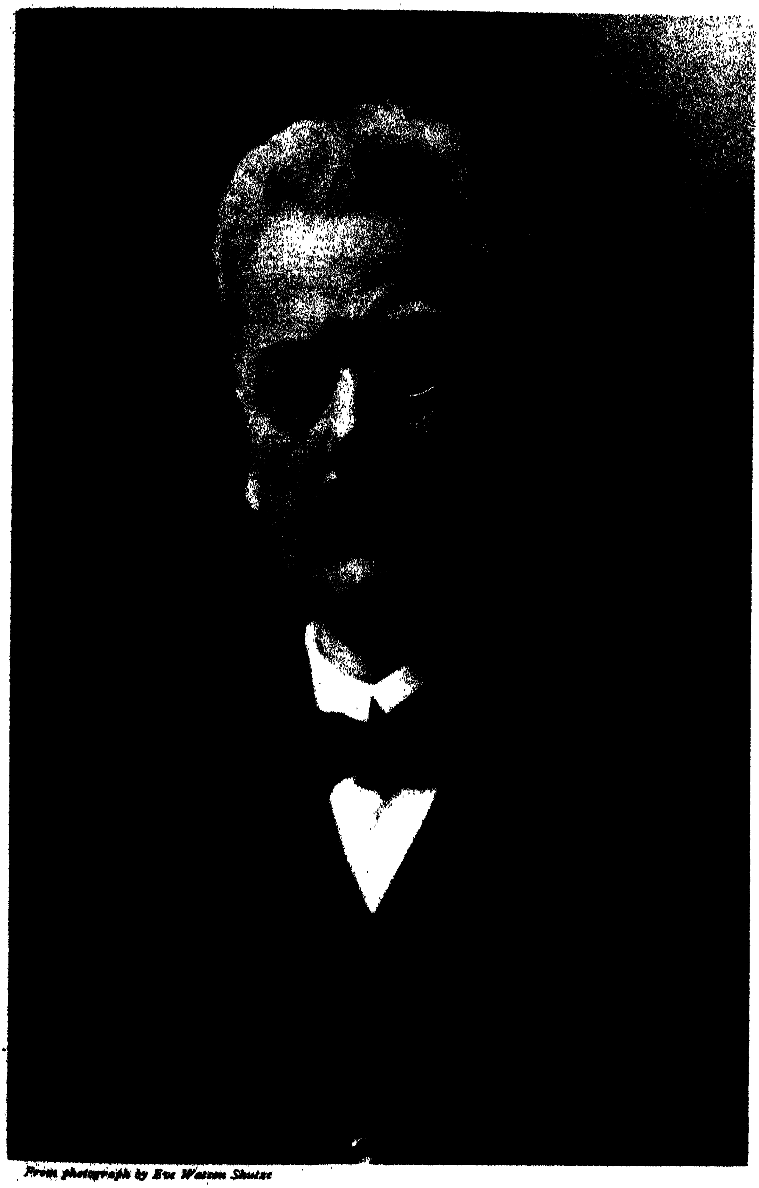
CHARLES FREDERICK MILLSPAUGH

(WITH PORTRAIT)

CHARLES FREDERICK MILLSPAUGH was born in Ithaca, New York, in 1854, and died at Chicago, Illinois, in 1923. As a child he displayed a strong interest in outdoor sports and in objects of natural history. It is related that in early boyhood he attracted the notice of the great LOUIS AGASSIZ when the latter was fishing near Ithaca, this chance meeting soon ripening into a lasting friendship between the two. A nephew on his maternal side of EZRA CORNELL, the founder of Cornell University, he spent the years 1872–75 as a student at Cornell. A few years later he went to the New York Homeopathic Medical College, where he was granted the degree of Doctor of Medicine in 1881.

For nine years following the receipt of his doctorate he practiced medicine in Binghamton, New York. Early during this period, however, he appears to have been lured by the charm of plant study. As with TORREY, GRAY, ENGELMANN, and so many other pioneers of American botany, his work along medical lines rapidly came to be eclipsed by an absorbing interest in plants. In 1887 he published his extensive work on *American Medicinal Plants*, one of the monumental American works in its line. This work was issued in ten volumes, with a total of one hundred-eighty full page colored plates delineated and painted by himself. Only those who have essayed to portray plants on such a comprehensive scale can appreciate the immense amount of labor that these plates represent. Their beauty and fidelity to nature are a credit, not only to the ability and accuracy of their artist, but also to the able instruction received from his father, JOHN HILL MILLSPAUGH, to whom he publicly expressed his indebtedness for whatever he might possess "of art in coloring and drawing."

In 1891–92 he was Professor of Botany at the University of West Virginia, forming an intimacy with the flora of that state which found expression in various papers, the most extended being his *Preliminary Catalogue of the Flora of West Virginia* (1891) and his *Flora of West Virginia* (Edit. I, 1896; Edit. II, 1913). In 1894 he was made Curator



From photograph by Eva Watson Shutze

CHARLES FREDERICK MILLSPAUGH

of Botany at the Field Museum of Natural History, Chicago, where he remained until his death. In rapid succession he produced various works of a taxonomic nature. These dealt chiefly with the North American Euphorbiaceae, the floras of Yucatan, the Bahama Islands, St. Croix and others of the West Indies, and Santa Catalina Island. Nor did he refrain from activities in the field. At various times he collected in different parts of the United States from Massachusetts to California, in the West Indies, Mexico, Brazil, and elsewhere. His plant labels at the Field Museum show that he made no less than four trips to Yucatan. In 1911–12 he made a journey around the world. Experienced at collecting and expert as a photographer, he returned with a vast number of specimens and photographs of scientific interest.

During his long and active botanical career many honors were bestowed upon him. He was Professorial Lecturer in Botany at the University of Chicago in 1895, and later was Associate Professor of Economic Botany at the same institution. In 1896 he was made Professor of Medical Botany at the Chicago Homeopathic Medical College. He was a Fellow of the American Association for the Advancement of Science, of the Faculdad de Medicina, Mexico, and the Faculdad de Medicina, Brazil; a member of the Torrey Botanical Club, the Explorers' Club of New York, Sigma Xi, and many other societies. In recent years he was a dominant figure in the American Wild Flower Preservation Society.

Toward the close of 1919, in an effort to recuperate his physical strength after a serious operation, he went to the Island of Santa Catalina, California. There he collected many specimens of plants, took numerous field notes and photographs, and laid the foundation for his *Flora of Santa Catalina Island*, which he published in collaboration with L. W. NUTTALL in January, 1923. This was his last published work.

The great herbarium and plant exhibits organized at the Field Museum under his personal direction will long pay eloquent tribute to his energy and enthusiasm. Probably nowhere else among the larger herbaria of the world are the plants so thoroughly indexed as to data of collection. During the past decade there have been made and installed at the Field Museum many beautiful and justly famous models of representative plants, which have proved at once the marvel and delight of visiting botanists from all quarters of the globe. He labored indefatigably to assemble at the Field Museum a great collection of valuable materials that some day should make Chicago a foremost center of taxonomic research.

To the younger generation of American botanists Dr. MILLSPAUGH was known mainly by reputation, for, busied with the many administrative tasks incident to his work, he often had to forego attendance upon conventions of fellow scientists. A full six feet in stature, of erect carriage and decisive manner, he possessed a strong and positive personality not soon to be forgotten by those who knew him. Accurately to appraise his various qualities and powers as a botanist would be well-nigh impossible, nor would the writer, biased with the warm friendship that comes from an acquaintance and close personal contact during the past twelve years, feel equal to such a task. True it is, however, that Dr. MILLSPAUGH's death marks the departure of an able taxonomic worker and one of America's most brilliant organizers of museum displays. —E. E. SHERFF.

USE OF NEGATIVE PRINTS FOR COMPARING MICROSCOPIC STRUCTURES

(WITH ONE FIGURE)

Recently the little used method of making photographs directly on sensitive paper has been found very satisfactory at the Forest Products Laboratory for obtaining enlarged photographs of wood structure for study.[1] Such photomicrographs made from very thin sections of wood, mounted in the usual manner, furnish permanent, easily available records of material investigated. They permit study and direct comparison of the minute structure at any desired magnification without the aid of a microscope. The photographs are negative of course, but this does not affect their usefulness; in fact, it has some advantages.

Permanent or temporary slide mounts may be used. In this case, permanent mounts of thin sections of wood, 10–20 $\mu\mu$ in thickness were photographed. These sections were colored with selective stains, using contrasting cardinal colors, such as reds and blues. As wood elements have an affinity for some colors with varying degrees of absorption, differential coloring was produced which showed as white, black, or intermediate grays in the photomicrographs.

In connection with a study on the occurrence of brashness in wood, it was found necessary to measure the areas occupied by the wood fibers on cross-sections of the wood in over one hundred microscopic sections of the wood of white oak. In the photomicrographs the fiber areas (white in the photographs) were clearly differentiated. An ordinary Dietzgen polar planimeter was used to measure these areas. Simple calculations gave the percentage of wood fiber area for each section.

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[1] Assistance in preparing photomicrographs was given by Dr. M. E. DIEMER, chemist and photographer at the Forest Products Laboratory.

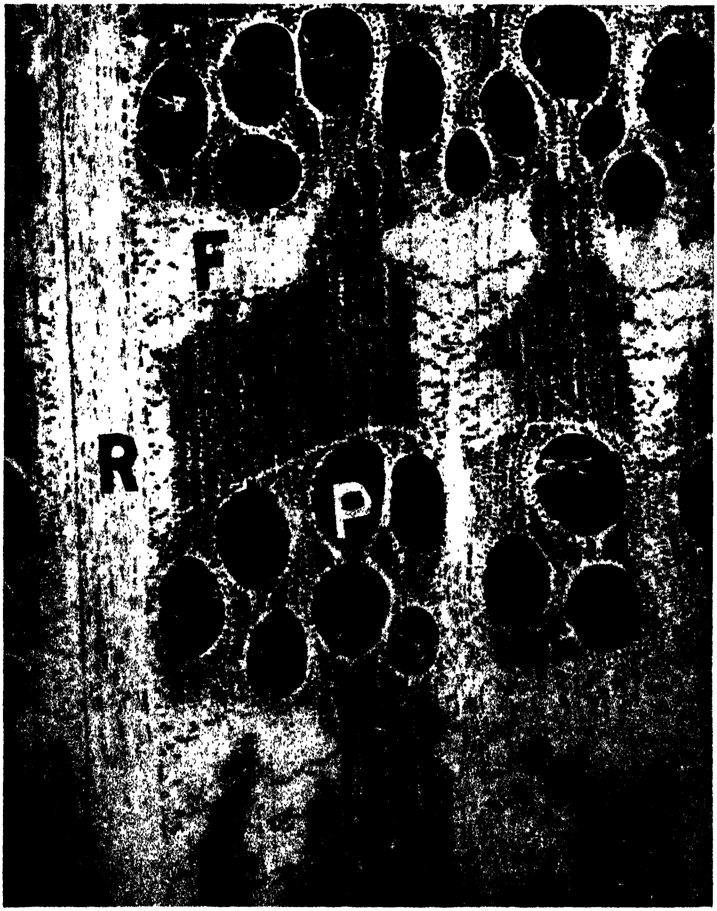

FIG. 1.—Negative print of cross-section through portion of two growth rings of white oak; *P*, pore; *R*, ray; *F*, wood fiber area.

Ordinarily, exposures are made on commercial dry plates or films from which later positives prints are obtained. The procedure adopted here dispenses with this step. It is inexpensive, a great time saver, and comparatively simple. The photomicrographic equipment used consisted of a carbon arc lamp on a 220-volt circuit, its housing, a microscope of the large-barrel type, a camera bellows, and focusing screen of ground glass. Only an objective is necessary with the microscope for lower magnifications. The equipment must be rigid and all parts in proper relative adjustment to secure sharp photographs. A long bellows is best, as it permits working wholly within the light circle and allows large plates to be covered.

Essentially, the process involves the making of negatives on ordinary photographic printing paper placed behind a clear glass in the plate holder. The exposure varies from 25 to 50 seconds, depending on intensity of light and thickness of wood sections. These negative prints are treated in the dark room in the same manner as paper which has been exposed behind the ordinary negative. For most work glossy prints are preferable.

Approximately the same conditions of light and shadow in the wood as are apparent to the eye are reproduced; that is, the pores are dark and the rays are light or white. Only the wood fiber areas are negative; they appear lighter than the surrounding tissues in the photograph, whereas on the end grain of a piece of oak they appear darker. The interchange of black and white by the use of negative prints is an advantage over the ordinary print in demonstration work, for the reason that the picture more nearly simulates actual conditions on viewing the end grain of the wood itself.—REUBEN W. SMITH, *Forest Products Laboratory, Madison, Wis.*

CURRENT LITERATURE

BOOK REVIEWS

Researches on fungi

The observations and experimental studies reported in BULLER'S *Researches on fungi*, published in 1909, have been continued, and so amplified that the original volume is now to be only the first of four. The second volume[1] has recently appeared, and the remaining two are said to be in an advanced stage of preparation for the press.

The study of the problem of spore discharge in the Hymenomycetes is continued. It is shown that each spore has a slight projection, the hilum, just above the point of attachment to its sterigma and pointing toward the central axis of the basidium. From the hilum, just before spore discharge, a tiny drop of fluid is extruded. This drop is always carried away with the spore. What changes occur in the spore and in the basidium at this time, and exactly how the violent spore discharge is brought about, remain to be discovered. The violent discharge itself is shown to occur not only in agarics, but in species belonging to the related families Polyporaceae, Clavariaceae, Hydnaceae, and Thelephoraceae, and also to the gelatinous families Tremellaceae, Dacryomycetaceae, and Auriculariaceae, and to the rusts, and is probably characteristic of all basidia borne in an exposed hymenium. Detailed discussion of the discharge in some of these groups is reserved for the future volumes. It is pointed out that the tremellaceous groups, in spite of their wide separation phylogenetically from the Hymeniales, have not only developed a similar mechanism for spore discharge, but have evolved pileate forms with spines, pores, and even gills, thus furnishing a striking illustration of phyletic convergence.

The major part of the volume is devoted to a careful analysis of the hymenium in agarics. Two distinct types are recognized: the non-*Coprinus* or Aequi-hymeniiferous type, and the *Coprinus* or Inaequi-hymeniiferous type. In the former type, which includes all genera of agarics except *Coprinus*, the gills are wedge-shaped in section and are positively geotropic; hence in a normally oriented hymenium, every part is directed toward the center of the earth. Spores are liberated from every part of the gills throughout the period of spore discharge, which may continue for several days, and each spore, on its discharge, is shot perpendicularly into the interlamellar space for a distance of 0.1–0.2 mm., after which its trajectory changes abruptly and it drops at a uniform rate to the outer air, where it becomes subject to air currents.

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[1] BULLER, A. H. R., Researches on fungi. Vol. II. Further investigations upon the production and liberation of spores in Hymenomycetes. 8vo. pp. xii+492. *figs. 157.* London: Longmans, Green, & Co. 1922.

In the *Coprinus* type of fruit body the gills are relatively thin, with sides almost or wholly parallel, and do not respond to the stimulus of gravity; hence, under natural conditions, part of the hymenium may be directed upward. The basidia are not matured simultaneously on all parts of the gills, but from below upward, so that all the basidia on a given level ripen their spores before the spores on the basidia above them have matured; hence the spores are discharged in regular succession, beginning at the bottom. As the zone from which the spores have been discharged advances from below upward on each gill, it is immediately dissolved by a process of autodigestion. The pileus flesh is thin and is also subjected to autodigestion after the spores have been discharged.

Each of these types is subdivided into several subtypes, and nearly half of the volume is devoted to a detailed consideration of the organization of the hymenium in *Panaeolus campanulatus*, *Anellaria separata*, and *Psalliota campestris*, all representing the *Panaeolus* subtype of the Aequi-hymeniiferae.

The author points out that no really satisfactory drawing of the hymenium, showing accurately the space relations of the hymenial elements one to another, has heretofore been published; that the behavior of a basidium after its spores are shed has never been followed; and that the distinction between basidia and paraphyses, and the rôle played by the latter elements, have been vague and uncertain. These deficiencies he supplies, publishing numerous beautiful illustrations and drawings, of which special reference should be made to figs. 96 and 147, illustrating the hymenium of *Panaeolus campanulatus* and of *Psalliota campestris* respectively. He shows that each basidium bears but one set of spores and then collapses, and that the paraphyses may, with ordinary care, be distinguished satisfactorily from the developing basidia. It is shown that as the basidia of successive generations collapse after discharging their spores, the paraphyses enlarge and become more and more prominent, thus keeping the hymenium turgid until the period of spore discharge, which may last for a week or more, is brought to its conclusion.

Other matters treated include xerophytism in tremelloid and non-tremelloid fruit bodies, variation in *Coprinus* and *Marasmius*, and squirrels and slugs as mycophagists. In keeping with its character, the book is notably free from typographical errors, and its one hundred fifty-seven illustrations are admirably selected, beautifully executed, and always pertinent.—G. W. MARTIN.

Saprolegniaceae

The results of many years of study of the water molds are brought together in a monograph by Coker[2] giving an account of all known species, with figures of all forms that he has observed in the living state. These illustrations cover 63 full page plates of excellent ink drawings, and constitute a very important part of the contribution, especially since there are shown for each species usually a number of stages in the life history. This is the first extensive treatment of this group of fungi as a whole since HUMPHREY'S monograph of 1893 and FISHER'S account of 1892 in RABENHORST'S *Flora.*

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[2] COKER, W. C., The Saprolegniaceae, with notes on other water molds. University of North Carolina Press. pp. 201. *pls. 63.* 1923.

Thirty years have added much of cytological and physiological interest, which shows itself in the character of the taxonomic treatment. For example, the description of *Saprolegnia ferax* with its synonymy, distribution, and references to other illustrations covers less than one and a half pages, but then follow five pages chiefly given to a discussion of the behavior of this species when grown under various cultural conditions. As regards taxonomic practice, it would appear that some species of water molds can with certainty be identified only after cultural tests.

Throughout the monograph there is frequent reference to the behavior of species when grown in various media. This is interesting reading, but one wishes that some general conclusions had been formulated and brought together on the effects of physiological conditions, since these seem to play so important a part in the expression of the plant's morphology. There are also numerous references to points of cytological interest in connection with particular species, but no general summary covering this matter for the group as a whole. The subject of chondriosomes receives considerable attention. The seasonal habits of a number of species are described and compared from extensive observations based chiefly on collections from the vicinity of Chapel Hall, North Carolina. In this more moderate climate there is no closed season, and water molds may be found in open water throughout the winter. This contrasts with the findings of PETERSON from studies in Denmark, where the colder winters generally confine the species to the months of spring, summer, and winter.

In all some 60 species are described from the author's personal studies, and quotations are given from descriptions of about 30 other species not known to America. Several pages and two plates, devoted to fungus parasites upon the water molds, will be welcomed by students of the group. The bibliography lists 234 titles.—B. M. DAVIS.

Heredity and chromosomes

A good translation into German makes available to a much wider constituency STOMPS'[3] recent work in Dutch on heredity and cytology. This is a readily intelligible account, and while no new ground is broken, the collection of material and the mode of presentation make it a useful book.

It is divided into three parts: the first, a treatise on the cell, cell division, and the reduction division; the second, entitled heredity, but really a short account of DE VRIES' intracellular pangenesis theory, with evidence regarding the functions of the nucleus; and the third, a more lengthy exposition of the relation of chromosomes and heredity. As regards cell division, the author claims that, owing to the accompanying vacuolization and the increase and decrease in vacuole size as regards nuclei, chromosomes, and protoplasm, mitosis may be regarded as an osmotic phenomenon. He considers that the pulling fibers may be permanently united between the chromosomes and the general protoplasm of the cell, although not visible in the resting stage. He holds with GATES that in the reduction division both telosynapsis and parasynapsis are possible. The material of the third part is presented chiefly in the form of an account of the classic researches of BOVERI and HERBST on sea urchins, and of MORGAN and BRIDGES on *Drosophila*. He considers that MORGAN'S work will convince all reasonable people that the physical basis of Mendelian properties in *Drosophila* is in the chromosomes, and that what is true of this species must be the case with all others. Mendelism itself receives scant attention and that near the close of the book. This treatment would appear to be both a defect of proportion and a cause of difficulty to readers unfamiliar with the subject.

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[3] STOMPS, THEO. J., Erblichkeit und Chromosomen, pp. viii+158. *figs. 24.* Jena: Gustav Fischer. 1923.

In general, however, the book is well written, and the historical method of presentation, which has been adopted throughout, has been cleverly utilized. It should prove very useful to those who require a simple, accurate, and up-to-date account of the chromosome theory of heredity.—R. O. EARL.

Plant biology

In a text book intended primarily for medical students and others who do not intend to continue the study of botany, TANSLEY[4] has arranged carefully selected material in a decidedly attractive manner.

After a brief introductory chapter, the author discusses organic substances and their chemical characters, and then the physical nature of organic substances, including the differences between crystaloids and colloids, gel formation, and the processes of diffusion and osmosis. The remainder of the book makes a survey of plant forms from *Protococcus* through the customary groups to the flowering plants. Type plants are taken with a view to using them as a basis for the discussion of biological principles. The student should have a fair understanding of such principles and the essential life processes of plants rather than detailed knowledge of plant structures and plant classification at the close of the book, which seems a very desirable result. The book is clearly and interestingly written, and seems thoroughly modern in the subject matter presented and the information imparted. Practical exercises are appended to each chapter. The book is by all means the best presentation of plant biology that has come to the attention of the reviewer. Used in conjunction with a corresponding book on elementary animal biology, as is the author's intention, it would make a very strong course. The zoölogical portion of the course at Cambridge covers the matter of heredity and evolution, which subjects are only briefly touched upon in this volume.—E. R. DOWNING.

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[4] TANSLEY, A. G., Elements of plant biology. London: George Allen & Unwin Ltd.; New York: Dodd, Mead & Co., pp. 410. *figs. 61.* 1922. $4.00.

NOTES FOR STUDENTS

Geotropism.—During the last few years a number of papers dealing with this phenomenon have appeared. CHOLODNYJ[5] has advanced a theory of geotropism in which he connects positive and negative geotropism with the existence in the cells of negatively charged microsomes. Being large particles, they tend under the influence of gravity to become more concentrated on the under side of the cell in the case of horizontally placed organs, thus causing the accumulation there of a greater negative charge of electricity. The negative charge attracts the metallic ions that are present, and they move to the lower part of each cell. Since the univalent ions migrate more rapidly than the bivalent, however, the ratio of univalent ions to bivalent ions changes, becoming smaller in the upper part of the cell, and larger in the under part. Reasoning from facts already established as to the colloidal and physiological effects of univalent and bivalent ions, the author draws a picture showing how this change in the ratio causes the two sides of the cell to differ in certain physical properties, and how permeability differences are set up, so that the under side of the cell grows faster than the upper. Thus negative geotropism results. In the case of positive geotropism, it is assumed that the microsomes are of different sizes, and that there is a sorting of them under the influence of gravity. This sets up a stronger electromotive force in the upper part of the cell. The theory is frankly advanced as a working hypothesis, and is evidently intended to stimulate research.

JACCARD[6] asks the question whether the unilateral growth of the trunks of trees, which results when a tree is displaced from its normal position with regard to gravity and which brings it back into its proper alignment with gravity, is the result of geotropic or phototropic stimulus perceived by the apex, and transmitted back to the growing region, or whether the stimulus results from the tensions and compressions which are caused by the inclining of the stem. JACCARD studied a number of cases of geotropic response, and comes to the conclusion that the tensions and compressions are the main cause of unilateral growth. He recognizes, however, the complexity of the problem, and admits that the orienting forces, gravity, radiation, etc., and other forces, have a part in determining the form of plants.

LYNN[7] is able to bring about a reversal of geotropic response of *Helianthus* seedlings, by subjecting them to various percentages of carbon dioxide, and offers this in support of SMALL'S[8] hydrion theory of geotropism.

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[5] CHOLODNYJ, N., Zur Theorie des Geotropismus. Beih. Bot. Centralb. **39**:222–230. 1922.

[6] JACCARD, M. P., Sur le mecanisme du redressement geotropique de la tige des arbres. Rev. Gen. Bot. **34**:433–441, 481–488, 529–537. 1922.

[7] LYNN, M. J., The reversal of geotropic response in the stem. I. The effects of various percentages of carbon dioxide. New Phytol. **20**:116–122. 1921.

[8] SMALL, J., A theory of geotropism with some experiments on the chemical reversal of geotropic response in stem and root. New Phytol. **19**:49–63. 1920.

Attempts to repeat this work with *Helianthus* have not been successful in producing reversal of normal geotropism, however, and the results reported by LYNN should be confirmed before placing reliance upon them. SCHULZ,[9] in the case of flowers, finds that the peduncles perceive and react geotropically. Removing the bud or any of its important parts, or wounding the bud destroys the normal action in response to gravity. Experiments with other plants are also reported. TRONDLE[10] shows that in the case of *Lepidium* radicles, the sine law holds without any limitation. DARWIN doubted whether fern fronds had the power to respond to gravity, but PRANKERD[11] shows that ferns have this power, and that the reason that DARWIN did not detect it was owing to their unusually long reaction time, and to the fact that they early lose their geotropic irritability.

This brief reference to a number of papers perhaps will show something of the nature of the present work in the field of geotropism. Our knowledge of it is still largely in the theoretical stage, and the experiments deal largely with superficial phases of the subject. With so much work going on, however, we should soon get back to some of the physical and chemical fundamentals of the phenomenon.—S. V. EATON.

Interspecific hybrids in Crepis.—A genus of many species, some of which have a low chromosome number and which may be crossed, forms promising material for an attack on some of the fundamental problems of botany: chromosome individuality, heredity, the evolution of chromosome number, and, in a limited way, the origin of species. Such a genus is *Crepis*, with species with 3, 4, 5, 6, 8, and 20 pairs of chromosomes respectively. In several of these a start has been made in the determination of specific hereditary characters. Recently, COLLINS and MANN[12] have obtained hybrids between a 3-pair and a 4-pair, and between a 4-pair and a 20-pair species.

C. capillaris has three pairs of chromosomes, one long, one short, and one intermediate. *C. setosa* has four pairs, one long with a semi-detached end, two intermediate, and one very short. In the F_1 seven chromosomes are found, the long capillaris, and the very short and the semi-detached setosas being readily distinguished.

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[9] SCHULZ, HELENE, Über Korrelationen zwischen den Blutenteilen und den geotropischen Bewegungen der Blutenschafte, nach Untersuchungen insbesondere an Papaver. Jahrb. Wiss. Bot. **60**:1–66. 1921.

[10] TRONDLE, A., Untersuchungen über das Sinusgesetz bei den geotropischen Reaktionen von Lepidium. Jahrb. Wiss. Bot. **60**:295–306. 1921.

[11] PRANKERD, T. L., On the irritability of the fronds of *Asplenium bulbiferum*, with special reference to graviperception. Proc. Roy. Soc. London B. **93**:143–152. 1922.

[12] COLLINS, J. L., and MANN, MARGARET C., Interspecific hybrids in *Crepis*. II. A preliminary report on the results of hybridizing *C. setosa* Hall. with C. *capillaris* (L.) Wallr. and with *C. biennis* L. Genetics **8**:212–232. *figs. 9.* 1923.

In somatic characters, *C. setosa* is predominant in nearly all respects. In the pollen mother cells the chromosomes are unpaired in diakinesis, and the first meiotic division is very irregular. Three, 4, 5, or 6 microspores may result, and no back crosses were obtained when the F_1 was used as the pollen parent. Some of the ovules are functional, however, and five plants were obtained on crossing back with *C. setosa.* Two had 10, two 8, and one 7 chromosomes in root tip cells. Only one of these has flowered, and here the reduction division occurs normally. Indeed the plant is typically *C. setosa* in general and in chromosome appearance. The plant with 7 chromosomes resembles the F_1 in every way. Although the authors make no mention of it, one would suspect apogamy here. Some interesting speculations are made as to the possibilities with the other forms. The results obtained are in general accord with similar work on such hybrids.

The *setosa*×*biennis* cross revealed some distinctly new phenomena. The disparity in chromosome number, 4–20, is surprising. Stranger still is it that the reduction division should be found so nearly normal. It has been considered that in hybrids between species of different chromosome numbers the maternal and paternal chromosomes always pair, leaving the extra chromosomes as univalents, but here as many as 10 bivalents are readily distinguished in diakinesis. It is evident, therefore, that since *C. setosa* contributes but four, those from *C. biennis* must have paired with one another. This indicates a qualitative similarity among what would be considered non-homologous chromosomes, and suggests that *C. biennis* is a polyploid species. The authors assume that it is octoploid, 5 being the basic number.

The univalents lag in the anaphase, and are irregularly distributed. F_2 plants have 24 or 25 chromosomes. It may be possible to produce stable 11-pair or 12-pair species. Back crosses with each parent have been obtained, from which it is hoped to obtain stable races with such haploid chromosome numbers as 10, 12, 14, 16, and 30. It would thus appear that breaks in chromosome number series may be caused by hybridization rather than by non-disjunction.

This report is remarkable as much for its speculations, which are in a fair way to be tested, as for its actual data. Further information will be awaited with interest.—R. O. EARL.

Aluminum and iron in relation to root rots.—HOFFER and CARR[13] have attempted to establish a connection between the root rots of corn and the presence of accumulations of aluminum and iron in the nodal tissues of the plants. Characteristic purplish brown discolorations of the vascular bundles at the nodes are not always associated with fungous infection, but can be produced artificially by injection of aluminum and iron salts into healthy plants, and are believed to be due to the accumulated metallic compounds.

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[13] HOFFER, G. N., and CARR, R. H. JR., Accumulation of aluminum and iron compounds in corn plants, and its probable relation to root rots. Jour. Agric. Res. 10:801–823. 1923.

Soils lacking in calcium and available phosphates are especially likely to produce root rotted corn, and the diseased stalks are found richer in iron and aluminum than the healthy stalks. Treatment of soils with acid phosphate helps to overcome the tendency to root rot, and potash is particularly valuable in preventing nodal injuries.

Various soils have been tested by HOFFER and TROST,[14] who report that the roots of plants grown in soils treated with aluminum chloride were very badly rotted by *Fusarium moniliforme.* They find that different strains of corn vary in their ability to absorb aluminum, and that strains which absorb the metals most freely are more susceptible to inoculations of root rot organisms.

The evidences for accumulation of iron in the nodes are drawn from qualitative colorometric tests, which are not very convincing. These observations should have been supported by macrochemical analyses which would have proved or disproved the presence of greater amounts of iron in the nodes of plants showing discolorations. Unpublished results of experiments conducted in the Hull Botanical Laboratory show that stalks infected with *Diplodia zeae* as the root rotting organism, always take up less aluminum than healthy plants.—J. C. IRELAND.

Nitrate and top-root growth ratios.—The effect of increased nitrate content of the nutrient solution upon the ratio of top growth to root growth has been investigated by TURNER,[15] who finds that in barley and corn the ratio of tops to roots is increased significantly with the increase in nitrate concentration of the nutrient solution. The effect is independent of total concentration, and hydrogen ion concentration of the solution. Flax, however, failed to show such an effect of nitrates. The nitrates do not retard root growth directly, but will increase the root growth if carbohydrates are present for the root nutrition with the nitrates. The effects noted are explained by supposing that the increased nitrate supply leads to greater use of carbohydrates by the tops, and this deprives the roots of part of their necessary carbohydrates. A relative reduction of root growth follows.—R. B. DUSTMAN.

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[14] HOFFER, G. N., and TROST, J. F., Accumulation of aluminum and iron compounds in corn plants, and its probable relation to root rots. II. Jour. Amer. Soc. Agron. 15:323–331. 1923.

[15] TURNER, J. W., Studies of the mechanism of the physiological effects of certain mineral salts in altering the ratio of top growth to root growth in seed plants. Amer. Jour. Bot. 9:415–445. 1922.

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ORIGIN OF PRAIRIES IN ILLINOIS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 314

JOHN WOODARD

The prairie province, which extends from Texas into Canada, covers the western part of Minnesota, but farther south extends eastward across Iowa, Illinois, and southern Wisconsin into the western part of Indiana. ATWATER (2) described a few isolated areas of prairie in Ohio, but nearly all of Ohio, as well as the greater part of Indiana, was forested when it was first settled by white men. The prairies extend almost down to the Ozark Hills in Illinois and almost to the Missouri River in central Missouri. South of the Missouri River they extend a short distance into southwest Missouri, but farther south they do not reach the eastern boundary of either Oklahoma or Texas. Some areas of prairie are found along the Gulf Coast in Louisiana and farther north in Mississippi and Alabama.

In the study of any prairie region, it is necessary to determine the conditions at the time of the prairie's origin which favored the invasion of a grass rather than a tree vegetation. As most of the prairies had their origin thousands of years ago, one can readily see that it is not easy to recognize all the conditions that influenced the course of plant succession. It is not necessary to assume that all prairies originated under the same conditions; in fact, it seems certain that they did not. The same effect may be the result of causes which differed more or less. It is necessary also to consider the conditions that have been in operation since the origin of the prairie. It does not necessarily hold that an area which has been occupied by prairie grasses will remain a prairie indefinitely. It is possible that the conditions in some places may not prevent the invasion of a prairie by forests, but may make the invasion very slow. Such places will remain as prairies for a much longer time than areas in which the conditions favor a more rapid invasion of prairie by forests. In a typical "grassland climate," such as is found in the main part of the prairie province, it is reasonable to suppose that the vegetation has reached a fairly static condition, and that there will be little change so long as the climate remains fairly constant. On the other hand, in Illinois, which is in a "woodland climate," it may be that the forests are invading the prairies, and that the prairies remain because the conditions prevent a rapid invasion, and the time since the origin of the prairies has been too brief for their complete occupation by forests.

Previous investigations have shown that prairies generally have a xerophytic vegetation; that the evaporation rate is much higher there than in adjoining forests, while the soil moisture content is much lower; and that, after the prairie is formed, it is difficult for tree seedlings to become established, except along forest borders and on eroded slopes. This paper reports an attempt to determine the conditions which favored the invasion of this region by grasses rather than by trees at the time the prairies were formed, and also attempts to determine the factors which have influenced the rate of invasion of forests into the prairies since their origin. "The actual cause of the treelessness of the prairies is not to be found in any of the present conditions" (GLEASON 15). Present conditions are important only as they help to interpret the past. A prairie is treeless because at the time of its origin the conditions favored the invasion of a grass rather than a tree vegetation, and the rate of forest invasion has been too slow for the complete occupation of the area by trees during the time which has elapsed since the prairie was formed. It is necessary to study present conditions, but it is also necessary to study past conditions. In this way only can one explain the present distribution of prairie and forest in Illinois and neighboring states.

The dominant prairie species are xerophytic grasses. This is shown by investigations of prairies in Illinois and in other parts of the world. The sand prairies along the Illinois River were studied by GLEASON (16) and VESTAL (41). Both investigators found xerophytic associations dominated by bunch grasses, among which were *Koeleria cristata*, *Leptoloma cognatum*, *Stipa spartea*, *Panicum pseudopubescens*, *Bouteloua hirsuta*, *B. curtipendula*, *Cyperus Schweinitzii*, *Andropogon scoparius*, and *A. furcatus*. VESTAL (40) studied a black soil prairie west of Chicago. He found two upland prairie grass associations, one dominated by *Andropogon scoparius* and the other by *A. furcatus*. The depressions were occupied by a marsh association, with *Liatris spicata*, *Eryngium yuccifolium*, *Spartina Michauxiana*, *Calamagrostis canadensis*, *Phragmites communis*, *Glyceria minata*, *Scirpus lineatus*, and *Typha latifolia*. SAMPSON (30) studied the prairie vegetation in all parts of Illinois:

> *Andropogon furcatus* is the climax grass of the whole upland prairie region of the state, and in the succession leading to this climax from the more xerophytic uplands and exposed clay soils *Andropogon scoparius* is the most important species.

According to SAMPSON, the sand prairie vegetation has a tendency to develop into the typical upland prairie vegetation with *Andropogon furcatus* as the dominant species, while the marsh grasses in the depressions are often replaced by *A. furcatus* when the depressions are drained.

In other parts of the prairie province, as well as in grassland regions in other parts of the world, the dominant plants are species of *Andropogon*, *Stipa*, or other xerophytic grasses. SCHAFFNER (31) lists *Andropogon furcatus* and *A. scoparius* as the two most important prairie grasses in Clay County, Kansas. HITCHCOCK (23) found *A. furcatus*, *A. scoparius*, and *Chrysopogon nutans* dominant on the prairies of eastern Kansas, while the more xerophytic *Buchloe dactyloides* was dominant in western Kansas. HARVEY (21) studied the prairie vegetation in southeastern South Dakota and found six dominant species: *Bouteloua oligostachya*, *B. curtipendula*, *B. hirsuta*, *Koeleria cristata*, *Andropogon furcatus*, and *A. scoparius*. SMITH (35) studied grazing conditions in the prairies of western Texas. He said the original vegetation was "largely blue stems and sage grasses (*Andropogon*)," and that these had been killed out by overgrazing and their places taken by other xerophytic grasses which were less desirable for pasture. HARPER (19) studied the vegetation on some Alabama prairies, and found the most abundant species was *Andropogon scoparius*. POUND and CLEMENTS (29) list a number of xerophytic grasses as dominant species in the prairie province. SHIMEK (33) found prairie plants growing on sand dunes, and emphasized the fact that these plants are xerophytes and can exist on dunes "as well as on the xerophytic surfaces of ordinary prairie." KOSSOWITSCH (24) found the steppes of Russia covered with xerophytic grasses, the most important of which are species of *Stipa*. SCHIMPER (32) describes the savannah as a region with xerophilous vegetation, of which "the majority of individual plants are grasses."

Prairies occupy areas where the irregularities of the surface are too slight to check the velocity of the wind to any great extent. The desiccating action of strong winds, especially the dry southwest winds, is very great. It is not surprising, therefore, that prairies have a high evaporation rate with correspondingly low soil moisture content during the latter part of the growing season. GLEASON and GATES (17) studied evaporation rates in *Quercus velutina* woods and in several stations in the sand prairies of central Illinois. The evaporation from a standard atmometer, which was placed in open ground where there were only a few weeds, was taken as 1, and the evaporation at the other stations recorded as ratios of the number. The evaporation rates at the prairie stations varied from 1.04 to 1.56, while the rate at the forest station was only 0.66. HARVEY (22) studied evaporation and soil moisture in a prairie near Chicago. He found the mean daily rate for the season higher than in the pine dunes, but lower than in the cottonwood dunes along the south shore of Lake Michigan. During the summer the soil moisture was low, often falling below the wilting coefficient. In Nebraska similar studies were carried out by WEAVER and THIEL (46), and by POOL, WEAVER, and JEAN (28). In both investigations a much higher evaporation rate was found on the prairies than in adjoining forests. The soil moisture content was much lower in the prairie than in the forest, falling below the wilting coefficient to a depth of two feet during part of the summer. WEAVER (45) found higher evaporation rates on southwestern slopes than on northeastern slopes in southeastern Washington. Some of the northeastern slopes were forested, while the southwestern slopes were treeless. In western Iowa, SHIMEK (33) observed similar relations between the evaporation rates and forest distribution. TODD (36) observed heavy timber on protected slopes, while there was little or none on exposed slopes in southwestern Iowa. VESTAL (43) found local inclusions of prairie within forest on slopes near the Embarrass River in the vicinity of Charleston, Illinois. These prairie areas are on the upper part of south-facing slopes where they are exposed to the desiccating effect of the southwest winds. HANSON (18) studied some prairie inclusions found in deciduous forests in Ohio, Illinois, Iowa, and eastern Nebraska. He found the evaporation rate much higher on the prairie than in the forests, while the soil moisture content was much lower in the prairies. In one of the forest stations the soil moisture content fell below the wilting coefficient once during the summer, but in the high prairie there was no water available for plants at four different times in the 0–10 cm. layer, and twice in the 10–30 cm. layer. Within forests evaporation is less in ravines than on the higher ground, as shown by TRANSEAU (37), FULLER (13), and ULLRICH (38). The evaporation rate seems to vary directly and the soil moisture content inversely with the wind velocity.

Tree seedlings have difficulty in becoming established on prairies because of the thick prairie sod and the desiccating action of the wind. Where artificial plantings are made, the trees grow and develop normally. Such an artificial planting in eastern Nebraska is described by POOL (27). In this grove the shade killed out the prairie grasses and they were replaced by mesophytic herbs. On the other hand, where forests have been destroyed by man, the mesophytic herbs die and xerophytic prairie grasses may replace them. SHIMEK (34) found a prairie vegetation along a road through a forest in Iowa. The destruction of the trees along the road exposed the mesophytic forest herbs to intense sunlight and strong winds, which killed them and allowed the invasion of the xerophytic grasses.

VESTAL (42) found a similar invasion of prairie grasses into a forest along the Big Four right of way near the Embarrass River in Illinois. Both of these areas would undoubtedly soon be reforested if man did not continually destroy the young tree growth. On the open prairie, however, the exposure is much greater, and prairie groves are rare. North of Charleston, in Coles County, Illinois, there is a small prairie grove which is on some low ridges at some distance from other timber. GATES (14) mentions isolated oaks on the body of the prairie, but thinks the invasion along forest borders, where the shade kills out the grasses, is much more important. SAMPSON (30) mentions four methods of invasion of prairies by forests:

> (1) Forest invasion accompanying erosion by streams, (2) growth of tree seedlings along forest borders where the grasses are checked by the shade of overhanging branches, (3) the occasional establishment of isolated seedlings farther out on the prairie, and (4) the growth of adventitious branch buds from the roots of certain trees and shrubs extending short distances into the prairie.

In western Texas, BRAY (5) found chaparral invading the prairie where the grass sod was destroyed by overgrazing. WELLS (47) reported forests on areas around St. Louis that were prairie when first settled by white men.

It is evident that prairies have a xerophytic vegetation because the wind velocity and evaporation rate are high while the soil moisture is low during the latter part of the growing season. Xerophytic grasses are able to make a rapid growth while there is sufficient water in the soil. They store food in their underground parts and go into a dormant condition during the dry weather. Tree seedlings are unable to compete with the grasses on the open prairie, but along the slopes of gullies and streams and some of the steeper moraines erosion removes the grass sod, and the irregularities of the surface check the wind velocity and reduce the evaporation, so that tree seedlings are able to secure a foothold. Trees can also advance slowly out on the prairie along the forest borders where shade kills out the grasses and the forest checks the wind velocity. The latter is very slow, so that the rate of advance depends to a large extent on the topography. It is necessary, therefore, to study the physiographic conditions in Illinois and neighboring states, for the differences in physiography are important in determining the present distribution of the prairies.

The Illinois prairies are postglacial in origin. According to CHAMBERLIN and SALISBURY (6), there were six different periods of glacial ice advance, which were separated by periods when the land was free from ice. The period between the Early and Late Wisconsin ice advances was relatively short, but all the other interglacial periods were very long. As each ice sheet advanced, it modified the surface of the country. As a general rule, the hills were leveled and the valleys filled, but in some places steep morainal ridges were formed by the glaciers. In this region, the surface left by the earliest glacier has been completely overrun by later ice advances, but all the other ice sheets left large areas where the surface has not been modified by later glacial advances. The Kansan ice sheet invaded northeastern Kansas and entered Missouri, extending as far south as the Missouri River. The Illinoisan ice sheet only entered the eastern edge of Iowa, but covered the greater part of Illinois, extending down to the Ozark Hills in the southern part of the state. Just east of the Illinois-Indiana line the border of the Illinois drift bends to the northeast, follows this direction to central Indiana, then turns south to the Ohio River, then east to south central Ohio, and then north to central Ohio, where it is buried by drift of the Wisconsin ice sheets. The Iowan ice covered northeastern Iowa and possibly a small area in northwestern Illinois. The Early Wisconsin glacier entered northeastern Illinois and extended as far south as Shelbyville and Mattoon, but in Indiana and western Ohio it extended almost to the border of the Illinoisan drift. A lobe of the Late Wisconsin ice sheet entered western Iowa, extending as far south as Des Moines. Another lobe entered northeastern Illinois, but did not extend as far to the west or south as the Early Wisconsin. Farther east, however, it covered nearly all of the Early Wisconsin drift in Indiana and western Ohio, and overrode all earlier drift sheets in eastern Ohio.

The glaciated region is bordered on the south by rough, hilly country from eastern Ohio to the western slopes of the Ozark Mountains in Missouri. Farther west, in the plains region, the relief is moderate. The Late Wisconsin drift has a very uneven surface, with many morainal ridges and depressions, but the Early Wisconsin, although it has some strong moraines, is nearly level over large areas in east central Illinois. The earlier drift sheets were originally nearly plane, but there must have been considerable pre-Wisconsin erosion near the larger streams, such as the Missouri, Mississippi, Rock, and Illinois Rivers. Much of the area, however, is still nearly level, as it was when the glaciers retreated. During the retreat of the last ice sheet a large lake, Lake Chicago, was formed in the southern end of the Lake Michigan basin, and another one, Lake Maumee, in the western end of the Lake Erie basin. Drainage to the north and east was blocked by the glacial ice, so that both of these lakes found outlets to the southwest, the former through the Chicago outlet and the Des Plaines River to the Illinois River, and the latter through the Wabash Valley to the Ohio River.

At the present time, large ice fields are bordered by treeless wastes, called tundras, where the vegetation is mainly mosses and lichens. The tundra varies in width in different regions and is often bordered on the south by forests. WARMING (44) says the tundra occupies only small areas in mountainous countries like Greenland, while it is very extensive in flat countries like northern Siberia. During the ice age conditions along the border of the ice fields must have been similar to those in Greenland and northern Siberia at the present time. The forest zone must have been separated from the ice front by a tundra zone which was narrow where the country was rough and hilly, but very wide where the country was comparatively level. During each ice advance, the tree line was depressed and the forests were "replaced by scrub, bog, and tundra" (CLEMENTS 7). Where the country was flat, the tree line was undoubtedly depressed for a great distance below the ice front, but where the country was rough and hilly, it is probable that, as pointed out by HARSHBERGER (20), "the glaciers did not affect the tree distribution at any great distance from the ice front." At the time of the maximum advance of the Late Wisconsin ice sheet, it is probable that trees were found only a short distance from the ice front in eastern Ohio, where the country is rough and hilly. In southwestern Ohio and southern Indiana the country is hilly, but it is not so rough as in eastern Ohio, so that the tundra zone was probably somewhat wider but still comparatively narrow. Farther west, however, where there was a wide belt of comparatively level country below the ice front, the tundra must have covered all the country as far south as the Ozark Hills in Illinois and the dissected bluffs along the Missouri River in Missouri, except the "driftless area" in northwestern Illinois and southwestern Wisconsin, and possibly some protected slopes along the larger streams such as the Mississippi, Rock, and Illinois Rivers.

Below the tundra, the rough, hilly country between the Appalachian Mountains and the west slopes of the Ozark Mountains was undoubtedly forested. Near the tundra the forests probably included both conifers and hardwoods, with the conifers on the exposed slopes and hilltops and the hardwoods along the protected slopes. In the plains region there were probably some protected slopes along some of the streams where trees were able to grow, and there may have been some depressions which were occupied by willows and tamaracks. As the greater part of the surface is gently rolling, however, tree seedlings would have been killed by the desiccating action of the strong winds. It is probable that the region as a whole was treeless, and that prairies bordered the tundra. At the time of the maximum advance of the Late Wisconsin ice sheet it is probable that the tundra, which was the first vegetation zone below the glacier, was narrow in Ohio and Indiana but very wide in the flat country farther west, extending almost to the southern end of Illinois and almost to the Missouri River in Missouri. South of the tundra there were undoubtedly forests extending from eastern Ohio to Missouri, while the country farther west was probably treeless plains covered by prairie grasses.

When the Late Wisconsin glacier began its retreat, it left a bare area that was invaded by the mosses and lichens of the tundra. These plants can endure the severe climatic conditions along the ice front, and can live on the pulverized rock material left by the glacier. Other plants were excluded by the severe climate and the inhospitable soil. As the ice retreated farther to the north the climate became less severe, and the tundra plants gradually added organic matter to the substratum and developed a soil suitable for the growth of the higher plants. According to Adams (1) the tundra was invaded by coniferous forests, and these by deciduous forests in the southeast and arid plains vegetation in the southwest. The actual condition, however, was not so simple as this. As already mentioned, it is doubtful whether there was any conifer belt along the tundra in the plains region. In this region, therefore, the prairie merged gradually into the tundra, and the latter must have been invaded by grasses from the former. Farther east, where forests joined the tundra on the south, both conifers and hardwoods moved northward, but their invasion was restricted to stream valleys and slopes, and to that part of the till sheet where there were considerable irregularities in the surface. On the level to gently rolling country, where there was no protection from the desiccating action of the strong winds, tree seedlings could not become established, and these areas must have been occupied by grasses. It seems probable that the prairie grasses, which invaded the tundra in the plains region, moved eastward, occupying all the country from which trees were excluded.

There may have been a postglacial period of aridity, but it is not necessary to assume such a condition in order to explain the origin of the prairies. Under the present moisture conditions, movement of forests into flat country is extremely slow. If the moisture conditions during all the years since the ice age were similar to what they are now, the northward movement of trees was very slow. If there was an arid postglacial period, the advance of the forests during that period was delayed, and may have been entirely prevented for a time, but it was resumed again when the climate became more moist. In either case, most of Illinois and Iowa, and large areas in northern Missouri, northern Indiana, and western Ohio must have developed from tundra into prairie. If there was an arid postglacial period, there may have been no invasion of the prairies by forests until the close of this arid period, but, if there was no such period, forest invasion began immediately, and has been going on slowly but steadily ever since the close of the ice age. The rate of invasion varies with the topography, and explains why some areas are completely forested while others are treeless prairies.

During the ice age the tundra, which covered most of Illinois and large areas in neighboring states, must have been similar to that found in the region west of Hudson Bay at the present time. HARSHBERGER (20) describes this region as follows:

> It is a treeless wilderness, low and marshy, interspersed with lakes, streams, and mossy plains, the so-called arctic tundra. The soil is permanently frozen

to a great depth, and in summer it thaws out a foot or two to permit the grasses, sedges, and other plants of the region to make a rapid vegetative growth.

Similar conditions probably prevailed in the tundra along the ice front during the Late Wisconsin glaciation. As the ice sheet retreated farther to the north, the summers gradually lengthened and the soil thawed to a greater depth. This permitted drainage of the higher ground, which was then invaded by the more xerophytic grasses, while the swamp grasses and sedges, which were the pioneer invaders of the tundra, were restricted to the depressions, which remained wet all summer. The invasion of the prairie grasses was probably rapid, and it seems likely that, early in post-glacial times, an immense prairie, with xerophytic grasses on the higher ground and swamp grasses in the depressions, had replaced most of the tundra in Iowa, Missouri, Illinois, Indiana, and Ohio. The grasses which grew on this prairie may have included the same species found on prairies today, and *Andropogon furcatus* may have been the dominant species as it is now on the prairies of Illinois.

As previously mentioned, the Late Wisconsin drift has an irregular surface with many morainal hills and depressions. When the ice retreated, the part of this drift sheet which is in Ohio and Indiana was separated from the forests on the south by a narrow strip of treeless tundra, while, farther west, there was a wide area of earlier drift which was nearly level and treeless. In this older drift, however, there were some large pre-Wisconsin stream valleys, such as the Mississippi, Rock, Illinois, and Wabash Rivers. The drainage waters from the glacial lakes for a time emptied into the Illinois and Wabash Rivers, and rapidly extended the valleys of these streams into the Late Wisconsin drift. These stream valleys were avenues for the rapid advance of forests.

This region was invaded by two types of forest vegetation, the upland and the bottomland. The latter followed the stream valleys, while the former advanced along the bluffs of the larger streams and in other places where the topography was rough. The upland pioneers were the pines, which early entered the rough country in northeastern Ohio and passed on into the morainal region of Ontario, where some of their descendants are still found. Conifers must have advanced rapidly along the Wabash, Illinois, Rock, and Mississippi Rivers. There are still white pine forests along the Rock

River which DE FOREST (12) thinks are relics of former more extensive pine forests. COWLES (10) mentions forests along the Illinois River at Starved Rock, where "the dominant tree vegetation is coniferous, consisting especially of the white pine (*Pinus Strobus*), and the arbor vitae (*Thuja occidentalis*)." As the pines are wind disseminated, it is probable that they advanced rapidly along these stream bluffs and spread rapidly in the rougher morainal regions of the Late Wisconsin drift. The pines were followed probably by the oaks, and these in some places by maples and beeches. In some places along the stream bluffs xerophytic shrubs instead of pines may have preceded the oaks.

Along the river bottoms the invading trees were probably the same species as found in similar situations today. COWLES (11) studied the vegetation along the Des Plaines River, where the pioneer trees are *Salix nigra* and *S. longifolia*. These are followed by *Acer dasycarpum*, *Populus monilifera*, and *Fraxinus americana*. When the bottomland becomes drier, *Ulmus americana*, *U. fulva*, *Tilia americana*, *Juglans nigra*, and *J. cinerea* appear. These or similar species must have moved north along the stream valleys at the close of the ice age. In the tundra region below the drift deposited by the Late Wisconsin ice sheet, there were stream valleys where invasion was rapid. In the Late Wisconsin drift, however, invasion of the bottomland vegetation must have been determined by the rate of stream erosion, except where there were depressions that were invaded by these trees. As the seeds of most of these species are wind disseminated, they are easily scattered, and it seems likely that seeds from trees growing along streams that were cutting back into the fresh till would fall along the shores of small lakes and ponds in some of the depressions. Seeds falling along these shores would undoubtedly germinate and in turn scatter their seeds to other depressions. As there were many of these depressions in the Late Wisconsin drift, it seems likely that they were an important factor in hastening the invasion of this drift by forests. Some of the depressions, which remained continually wet because they were deep or fed by springs, developed into bogs. These bogs have been invaded by trees, and some of them entirely, but others only partially, occupied by forests.

Trees not only invaded the bottomlands and river bluffs, but they also moved up over the crest of the bluff and slowly moved out on the prairie. This invasion, as already noted, is very slow. In this invasion of the prairies it is probable that the oaks were pioneers, or xerophytic shrubs were the pioneers followed by the oaks. Where the bluffs are not dissected by tributary streams and gullies, the forest advance has been extremely slow, and there is only a very narrow belt of timber along the bluff. Where there has been considerable erosion, however, the forest belts are much wider. Where the gullies are close together, forests have often occupied the intervening area, but in some places narrow strips of prairie are still found along the tops of the ridges between the gullies. Where erosion has cut deep ravines, the vegetation quickly reaches the mesophytic forest stage. Near Charleston, Illinois, where the Embarrass River cuts through the Shelbyville moraine, there are many deep ravines which have an almost pure stand of *Acer saccharum*. Similar mesophytic conditions in ravines in the Chicagò region have been described by COWLES (11). Where streams cut through moraines, trees not only occupy the slopes along the gullies, but they extend out along the slopes of the moraines beyond the heads of the gullies. This has been observed along the Shelbyville moraine near the Embarrass and Kaskaskia Rivers. In some places, especially along streams that are cutting back into flat prairies, erosion is not very deep, and the gullies extend only a short distance from the streams. These gullies are xerophytic, and it is probable that they were first invaded by xerophytic shrubs followed by oaks.

During the ice age the level uplands for a long distance below the ice front must have been free from tree vegetation. Trees, however, could grow along some of the stream bluffs and bottomlands. As the evaporation was less during the cold glacial period than during the warmer postglacial period, trees may have invaded stream valleys in the plains region from which they are now excluded. BESSEY (3) describes a deep canyon in western Nebraska where he found *Pinus ponderosa* var. *scopulorum*, *Juglans nigra*, and *Ostrya virginica*. These trees may be relics of vegetation which extended all along the streams during the glacial period, but was unable to endure the greater postglacial evaporation in the more exposed situations.

As already mentioned, after the retreat of the last ice sheet trees moved northward, along the stream valleys, the shores of lakes and ponds, and the steeper morainal and preglacial ridges. After these areas were occupied, the trees slowly invaded the more level intervening areas. Where these intervening areas were small, they have been mainly or entirely occupied by the forests, but where they are large, a considerable part of them is still treeless. In eastern Ohio, where there are some preglacial ridges and many strong moraines, all the country was forested except a few bogs, which were only partially timbered, like the one in Trumbull County. A large part of western Ohio is comparatively level, but in this region the soil map of Ohio by COFFEY and RICE (9) shows many areas of soils that developed in timbered swamps. Large areas of these soils are found in the bed of glacial Lake Maumee and in the level areas between the larger streams. Smaller areas are scattered all over the till plain in western Ohio, and there are undoubtedly more which were too small to be shown on the map. If, as seems probable, these depressions were invaded by trees in early postglacial times, it is not surprising that the intervening areas have been occupied by forests except for a few comparatively small areas like those mentioned by ATWATER (2). In eastern Indiana the conditions are similar to those in western Ohio, but in western Indiana there is more flat country, and consequently a large area of prairie. This Indiana prairie is continuous with the Illinois prairies, as shown by COFFEY (8), who separated the soils in this region into dark colored prairie soils and light colored timbered soils.

In western Indiana, Illinois, Iowa, and northern Missouri, pre-Wisconsin erosion had dissected some of the country along the Wabash, Illinois, Rock, Mississippi, and Missouri Rivers. Between these streams the country south of the Late Wisconsin drift is comparatively level. These flat areas have few depressions, so that the invasion of forests has been almost entirely along streams and gullies, and the slow advance along the forest border. In southern Illinois forests extended farther out into the flat prairies than they did in central and northwestern Illinois, because invasion began much sooner in southern Illinois than farther north. In northeastern Illinois, however, where the Late Wisconsin glacier left an uneven surface, forests have advanced out on many of the moraines between the streams, although the time has been much shorter. During the Late Wisconsin glaciation the tundra probably extended to the rough country in southern and eastern Williamson County, Illinois. The flat country in the central and northwestern parts of this county must have had a tundra vegetation which was later invaded by the prairie, a few small remnants of which still remain as prairie. In Franklin and Perry Counties more of the inter-stream areas is still prairie, while in Champaign County the forests occupy only narrow strips along the streams. Along some streams there are isolated areas of forests where the bluffs are eroded, while the gentle slopes are treeless. Low stream banks are often treeless for long distances, but steep bluffs are almost always timbered. These differences have been observed in different parts of Illinois, and similar differences in other parts of the state have been shown by the work of the Illinois Soil Survey (39).

MOSIER early recognized the differences between the prairie and forest soils, and in his classification the soils are grouped into prairie and timbered soils. These differences make it possible to map the boundary between forest and prairie in places where the forests have been cleared and both forest and prairie are now under cultivation. The Illinois soil maps, therefore, show the location of prairie and forest, and when the work is completed, they will show the distribution of prairie and forest in all parts of the state. COFFEY'S (8) map is valuable in showing the distribution of prairie and forest in the United States as a whole, but he grouped the gray prairie soils of southern Illinois with the timbered soils because he considered the color more important, from the soil standpoint, than the vegetation. His map, therefore, does not show all the prairies in Illinois. As topography is an important factor in the classification of the timbered soils in Illinois, the maps give considerable information on the topography of the forested areas. The yellow silt loams and the yellow sandy loams occupy rough eroded areas. They are found on morainal and preglacial ridges where the slopes are steep, on bluffs, and in areas that are badly gullied. In some places these eroded soils are found in narrow strips along bluffs, and in narrow tongues along gullies extending back into other timbered soils or even into the prairies. In other areas these soils occupy broad belts with jagged edges. The points along the jagged border are the heads of gullies that are separated by narrow steep ridges. The yellow gray silt loams and the yellow gray sandy loams vary in topography from slightly less rough than the eroded soils to almost level. They are found in areas of varying width bordering the eroded soils, along streams where the bluffs are low and the slopes too gentle for rapid erosion, and on moraines and preglacial ridges with gentle slopes. Flat or nearly level areas with an impervious clay subsoil are mapped as light gray silt loam on tight clay, white silt loam on tight clay, or yellow gray silt loam on tight clay. These soils are found mainly in the southern part of the state, and represent areas that were formerly prairie soils, either gray silt loam on tight clay or brown gray silt loam on tight clay. The timber (mainly oaks) has changed the character of the soil, particularly the color of the surface soil, which is a lighter gray than in the prairie soils from which they were derived. The soil maps of Bond and Clay Counties show that a large part of these soils is between streams and gullies where the invading trees advanced from opposite directions. Some of the light gray silt loam on tight clay in these counties is along the edge of the prairies, and shows that the trees have been able to invade the flat prairie for some distance. As these areas are in the southern part of the state and are near the Wabash and Kaskaskia Rivers, the time for this invasion must have been very long. In these counties there are some small timbered ridges surrounded by prairie and some areas of prairie surrounded by timber. Farther north, in Moultrie County, where there is less erosion and the forest invasion began at a much later date, the forests extended only a short distance from the streams. Adams County, which lies between the Mississippi and Illinois Rivers, is badly dissected, and the greater part is timbered. Many narrow strips of prairie are found on the tops of ridges between the streams and gullies. In Lake, Kane, and DuPage Counties, which are in the Late Wisconsin glaciation, a large part of the irregular surface is forested.

All the evidence indicates a once more extensive prairie region in Illinois and the neighboring states, which has gradually been reduced in area by the invasion of trees. Fires probably have retarded the advance of the forests, but they are not responsible for the treelessness of the prairies (BOURNE 4). Where forests have been destroyed locally by fires, it is doubtful whether the area is ever occupied by prairie grasses. Such areas are generally invaded by fire weeds followed by brambles and then trees. The idea of LESQUEREUX (25), that "all the prairies of the Mississippi valley have been formed by the slow recess of sheets of water of various extent, first transformed into swamps and by and by drained and dried," is also untenable. McGUIRE (26) suggested that the Alabama prairies once constituted the boundary of the Atlantic Ocean, and it may be that these prairies, as well as some others in unglaciated regions, are relics of the grass vegetation which first invaded the bare area left by the withdrawal of the oceanic waters. The prairies of Illinois and neighboring states, however, are in a region that was covered by glacial ice during the Pleistocene Period. At one time geologists believed the loess was a water deposit. If such had been the case, it would be reasonable to suppose that the prairies arose on the recession of the waters that deposited the loess. There is no evidence, however, that all, or even a large part of Illinois and the neighboring states was ever covered by water since the ice age. The loess is now believed to be wind deposited, and the prairies found on these loess deposits could hardly have arisen from swamps that were formed on the recession of water. During the ice age, there must have been a tundra along the border of the ice, just as there is along the borders of ice sheets at the present time. It seems likely that this tundra covered all of Illinois where prairies are now found, and that the tundra was invaded by prairie grasses when the glacier receded. As postglacial time has been too short for the complete occupation of this region by forests, part of Illinois is still prairie, but it is probable that all of Illinois would eventually have been forested, if man had not put a stop to all natural succession. The rate of invasion of flat prairies is very slow, however, and it would take a very long time for the forests to completely occupy these areas.

Summary

1. The prairies of Illinois are near the eastern edge of an eastern extension of the prairie province, and are really in a region with a "woodland climate."

2. Previous investigations have shown that prairies generally have a xerophytic vegetation, a high evaporation rate, and a low soil moisture content during part of the growing season; and that tree seedlings are generally excluded from the body of the prairie, but are able to invade the prairie slowly along the borders of forests and more rapidly along streams and ridge slopes.

3. Most of Illinois is covered by glacial drift which was deposited during several ice advances. The earlier glacial deposits, which cover most of the glaciated area in Illinois, were originally comparatively level, while the last drift sheet, which entered northeastern Illinois and covered most of the states to the north and east, has an irregular surface with many moraines and depressions. Part of the earlier drift was eroded by large streams before the advance of the last ice sheet. The glacial lakes, Chicago and Maumee, excavated outlet channels through the last drift sheet.

4. During the ice age there must have been a tundra along the border of the ice, just as there is in the Arctic regions at the present time. This tundra was probaby narrow in Ohio and Indiana, but very wide in the level country farther west, extending nearly to the southern end of Illinois and nearly to the Missouri River in Missouri. South of the tundra there were undoubtedly forests in the rough country, which extended from Ohio to Missouri, but treeless prairies in the level plains region farther west.

5. When the last ice sheet retreated, the bare area which it left was invaded by the tundra, and the tundra was invaded by other plants. In the plains region prairie grasses invaded the adjoining tundra, and moved east, occupying all the level country, from which trees were excluded by the desiccating action of the strong winds. It is probable that all the level country as far east as Ohio became prairie early in postglacial times. Forests invaded the hilly uplands and also along the stream valleys, and then spread to the shores of small lakes. After the forests occupied these areas they gradually moved out into the intervening areas.

6. In Ohio and eastern Indiana morainal hills and depressions are numerous and the intervening areas comparatively small, so that only a few small prairies remain in this region. Farther west, however, in western Indiana and Illinois, the flat inter-stream areas are larger, and many of them still remain as prairies, although forest trees have moved north along the larger stream valleys into the rougher country of Michigan and Wisconsin, which were almost completely forested.

7. The Illinois soil classification groups the soil types into prairie and timbered types, so that the soil maps of this state show the distribution of prairie and forest and the relation between stream dissection and forest invasion. The published maps show some invasion of moraines by the forests in northeastern Illinois, but in other parts of the state forest invasion has been confined almost exclusively to the slopes of streams and gullies and some of the adjoining level land.

8. Fires may have checked the invasion of forests into prairies, but prairies never originated by the destruction of forests by fires. Some unglaciated regions may have prairies that are relics of the vegetation that invaded the land when it first appeared above sea level, but the Illinois prairies are in a glaciated region, and there is no evidence that this region was covered by water at any time since the ice age. During the glacial period the prairie region of Illinois and neighboring states was probably occupied by a tundra which was invaded by prairie grasses. The prairie has been invaded by forests, and most of the states to the east and north of Illinois have been forested, but large areas in Illinois are still prairie, because postglacial time has been too short for the forests to invade such large areas of level land.

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TEMPERATURE COEFFICIENT OF ABSORPTION IN SEEDS OF CORN

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(WITH ONE FIGURE)

Introduction

In a previous paper (17) dealing with the temperature coefficient of moisture absorption in seeds, it was shown that the curves of water intake for cocklebur seeds and for cotyledons of peas removed from their coats could be represented by one or a series of logarithmic equations having the general formula $y=a \log (bx+1)+c$. In this equation, y is the total intake at any moment, x is the time during which absorption has proceeded, and a, b, and c are constants. The velocity of water intake may then be represented by the formula $v=ae^{-k\phi}$, in which ϕ is the previous absorption. The velocity of water intake is therefore not an exponential function of the temperature, but an inverse exponential function of the amount of water previously absorbed. The value of the temperature coefficient, Q_{10}, varied in different tests from 1.55 to 1.83, values too low to support the theory that the rate of water absorption depends solely upon a chemical phenomenon, such as the simplification of associated water molecules as the temperature rises. Since it was found that the temperature coefficient was about the same whether or not a semipermeable membrane was present, it appears that semipermeability per se may not be an important factor in determining the rate of permeation of water through such membranes.

The original work on temperature and absorption was done by BROWN and WORLEY (6) on *Hordeum* seeds. Since none of the seeds used in the previous work belonged to the same family of plants as barley, and represented fatty and protein seeds rather than carbohydrate-high seeds, it was felt that the study should be continued with some member of the Gramineae. After several kinds were tested, corn was chosen because of certain obvious advantages which it possesses. It has a tough coat, not easily ruptured, the coat can be removed readily, and is suited to certain membrane studies which are in progress. No other common member of the family offers such favorable material for direct permeability investigations.

Some preliminary experiments on corn were made by students at the University of Kansas, beginning in 1914. During the summer of that year BUSHNELL studied the influence of temperature and salts on the rate of water absorption in Leaming yellow dent corn, and was able to show indirectly that corn grains have osmotically active coats. It was apparent from the data, however, that the caryopsis coat is not as efficient in excluding NaCl and LiCl from the interior of the seed as is the seed coat of *Xanthium*. Before undertaking the collection of the data to be presented later, an attempt was made to analyze the data collected by BUSHNELL. Irregularities undoubtedly beyond the control of the investigator made the data useless for our purposes, and new series covering the range of BUSHNELL'S work were run with the greatest care.

A little later LOVEJOY removed the membrane from corn grains and used it in an osmometer similar to the one used by SHULL (16), and was able to confirm by direct measurements the indirect findings of BUSHNELL. The coats gave very good osmotic action, but were not entirely impermeable to the solutes used.

The temperature studies were resumed at the University of Kentucky, and have been concluded in the Hull Botanical Laboratory of the University of Chicago.

Materials and methods

In the later work, a variety of corn known as Hickory King has been used. It is a white dent corn with broad shallow grains and fairly hard endosperm. The breadth of the grains gives an excellent membrane for permeability studies. Attention has not been confined to this material, however, for even a casual survey of absorption phenomena shows that each individual kind of substance has its own peculiar water absorption complex. The physical structure or chemical composition of a material may so affect its water relations during absorption that mathematical treatment of the data is precluded. In some cases the intake of water, as determined by successive weighings, is not so much a measure of the absorption of water as it is the measure of some other physical or chemical process. To illustrate these irregularities of absorption, a variety of substances has been used, including seeds, cherry wood blocks, and tissue from *Auricularia auricula-judae*, one of the Protobasidiomycetes whose gelatinous body is admirably adapted to such studies. Some of these irregular cases will be considered in connection with the general discussion, without attempt to present the heterogeneous data, which are not needed for a general understanding of the situation.

The methods of securing data, and of analyzing them, are the same as those used in a former study (17). Every possible precaution was taken to produce and maintain uniform conditions during the time periods of intake, and to reduce the inherent errors to a minimum. The value of taking such precautions is seen in the uniformity of the main intake curves. The data for analysis were selected from a large series of determinations, the selection being based on the absence of visible internal cracking or other evidence of abnormal behavior, and without any attempt to study the data mathematically before selection. It was felt that this kind of selection was justifiable, for when some sharp internal physical disarrangement of the seed occurs, such as cracking of the endosperm, the series in which it occurs is so irregular that it offers insuperable difficulties to the mathematical treatment.

As absorption in corn is much slower than in some other types of seeds, and the seed is so large that saturation is a matter of many hours, no attempt was made to carry the curves farther than 20 per cent intake. This affords six tangent measurements, each 2.5 per cent apart, running from 7.5 to 20 per cent, a sufficient number to give satisfactory values for Q_{10}, the coefficient for a 10° rise, over the range of these experiments. In order to lessen the labor involved in the analyses, the curves have not been run through the point of origin, but start at some point below 7.5 per cent of intake. The temperature range covered is much wider than in the earlier work, and provides a crucial test of the theory that absorption is dominated as to rate by a chemical process. Beginning at 5° C., data are presented for each 10° interval up to 55° C., a range wide enough for all practical purposes.

Experimental results

Only the data which have been used in the mathematical analyses need to be recorded here, one series for each temperature. The percentage of intake, with time, for six different temperatures, is shown in table I. The calculations are all based on the air dry weight of the seeds. The graphic presentation of the data is shown in fig. 1, from which the effects of temperature on absorption rates can be seen at a glance. The curves are all fairly regular, except the curve for 15° C., which for some reason runs low in the early part of the curve, and somewhat high in the later portion. This irregular formation for this curve modifies the ratios of absorption rates for the curves immediately above and below. This situation will be referred to in the presentation of the mathematical analysis of the data, which follows.

TABLE I

ABSORPTION OF WATER BY CORN IN PERCENTAGE OF AIR DRY WEIGHT

Time in minutes	Temperature					
	5°	15°	25°	35°	45°	55°
1	1.58	1.46	2.04	1.89	2.01	2.12
5	3.29	3.17	3.58	3.81	3.79	4.16
10	4.06	3.86	4.58	4.94	4.86	5.80
15	4.56	4.34	4.96	5.76	6.04	6.92
30	5.23	5.27	6.04	7.10	7.95	9.46
45	5.79	5.99	6.97	8.34	9.53	11.40
60	6.40	6.69	7.78	9.31	11.12	13.18
90	7.24	7.50	9.09	11.09	13.26	16.30
120	7.92	8.26	10.36	12.85	15.38	18.75
180	9.23	9.81	12.70	15.67	18.99	23.21
240	10.25	11.33	14.40	17.95	21.99	....
300	11.26	12.89	16.06	19.87	24.24	....
420	13.10	15.76	19.15	23.21	27.80	....
540	14.55	17.83	21.67	....	....	....
660	16.03	19.75	....	....	....	....
780	17.35	....	....	....	....	....

Mathematical analysis

The rate of moisture intake at any given moment can be calculated very accurately from the tangent of the absorption curve at the time chosen. The tangent itself, however, cannot be measured very accurately from an ordinary graph. In order to measure the velocity of absorption with accuracy, it was first necessary to construct mathematical curves of known form, which would follow the empirical data of table I as closely as possible. Then points of equal intake were chosen on each temperature curve, the tangents calculated from the formula, and the velocity of intake computed from the tangents.

The equation mentioned in the introduction serves admirably for the construction of these mathematical curves through the corn data, although it was developed in connection with the work on cockleburs and pea cotyledons. Indeed, one seldom finds biological data that approach the mathematical accuracy shown by these water absorption curves for corn. By confining attention to the main curve of intake (omitting initial intake, and stopping before saturation effects were manifest), it has been possible to express each temperature curve by a single equation. It is quite probable that the initial intake curve and the approaching saturation curve could be followed by the same type of curve, with different constants, and with common tangents at the junction points of the curves. The procedure is tedious, however, and the analysis would add little to the interpretation of the process of absorption. This phase of the problem was adequately considered in the paper cited. The six equations for the six temperatures used, with numerical values substituted for the constants *a*, *b*, and *c*, are shown in table II.

TABLE II

FORMULAS OF CALCULATED CURVES

Temperature	Formula
5	$y=29.7\log_{10}(0.0020x+1)+5.19$
15	$y=60.4\log_{10}(0.0012x+1)+4.75$
25	$y=29.6\log_{10}(0.0051x+1)+4.28$
35	$y=29.6\log_{10}(0.0078x+1)+4.38$
45	$y=32.5\log_{10}(0.0106x+1)+4.00$
55	$y=34.5\log_{10}(0.0140x+1)+4.06$

The abnormality of the 15° curve mentioned in connection with the data is seen here, in the constants which were required to match the empirical data. Its disagreement with the other members of the family of curves will be seen more clearly in the intake ratios.

The extreme closeness with which the curves calculated from these six equations follow the experimental data of table I is shown in table III, where the calculated percentage of intake is given for each time interval; the amount of disagreement with the actual data is indicated, with a plus or minus sign to show whether the data are above or below the calculated intake. The columns indicating the discrepancy between the calculated intake of water and the actual intake observed are worthy of note. The largest difference between the theoretical and observed absorption is less than one-half per cent, and the average difference is only one-tenth of one per cent. Such close agreement is very remarkable for biological data.

TABLE III

INTAKE CALCULATED FROM FORMULAS, WITH DISCREPANCY OF DATA

TIME IN MINUTES	TEMPERATURE											
	5°		15°		25°		35°		45°		55°	
	Calculated	Data	Calculated	Data	Calculated	Data	Calculated	Data	Calculated	Data	Calculated	Data
10..........	......	......	......	......	......	......	......	......	......	......	6.02	−0.22
15..........	......	......	......	......	5.23	−0 27	5.80	−0.04	6.08	−0.04	6.92	0.00
30..........	......	......	5.68	−0 41	6.11	−0.07	7.08	+0.02	7.90	+0.05	9.31	+0.15
45..........	......	......	6.13	−0.14	6.94	+0 03	8.25	+0.09	9.50	+0.03	11.38	+0.02
60..........	6.65	−0.25	6.57	+0.12	7.71	+0.07	9.32	−0.01	10.95	+0.17	13.20	−0.02
90..........	7.32	−0.08	7.44	+0.06	9.13	−0.04	11.22	−0.13	13.46	−0.20	16.28	+0.02
120..........	7.96	−0.04	8.28	−0 02	10.42	−0.06	12.87	−0.02	15.58	−0.20	18.83	−0.08
180..........	9.16	+0.07	9.88	−0 07	12.65	+0.05	15 66	+0.01	19.07	−0.08	22.93	+0.28
240..........	10.25	0.00	11.39	−0.06	14.56	−0.16	17.94	+0.01	21.86	+0.13	......	......
300..........	11.25	+0.01	12.82	+0 07	16.21	−0.15	19.88	−0.01	24.19	+0.05	......	......
420..........	13.06	+0.04	15.46	+0.30	19.00	+0.15	23.06	+0.15	27.94	−0.14	......	......
540..........	14.60	−0.05	17.85	−0.02	21.29	+0.38	......	......	......	......	......	......
660..........	16.04	−0.01	20 05	−0.30	......	......	......	......	......	......	......	......
780..........	17.31	+0.04	......	......	......	......	......	......	......	......	......	......

In order to measure the absorption rates under equal conditions within the seeds, it is essential to compare velocities of absorption at a time when the seeds have absorbed equal quantities of water above the air dry condition. By reference to fig. 1, it will be seen that six levels of intake have been chosen for tangent measurements. These are indicated by the horizontal lines, where the intake, y, is 7.5, 10, 12.5, 15, 17.5, and 20 per cent above the air dry condition of the seed. In table IV is shown the length of time in minutes required to reach each level of intake at which the tangents were measured, together with the rate of intake at the moment when the curves pass the tangent points. The rates of absorption per minute are expressed in percentages of the original dry weight of the seed. Table IV shows how the time required to reach a given stage of absorption is decreased by rising temperature, and the intake rates show how the rate falls off with increasing absorption at any given temperature.

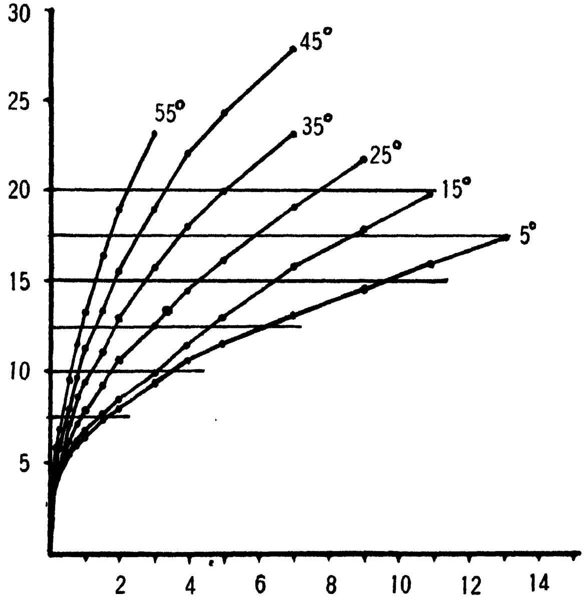

FIG. 1.—Absorption curves for corn at various temperatures: abscissae, time in hours; ordinates, percentage of water intake.

From the intake velocities of table IV, the ratios of the velocities at equal intake levels for 10° differences have been computed. These ratios are the temperature coefficients of absorption, Q_{10}, and are presented in detail in table V.

The average value of Q_{10} (the average of the averages for each series at the bottom of the table) is 1.537, which is very slightly less than the 1.55 obtained in the case of *Xanthium* seeds several years ago. The rapidly increasing ratios in the column 15°/5°, and the correspondingly falling series of ratios 25°/15°, are caused by the peculiarities of the 15° curve, referred to earlier. The intake was relatively too slow during the earlier phases of intake, and too rapid later on to fit properly between the 5° and 25° curves. Inspection of the two series shows that the 15° curve passed through its normal position at an intake of about 11 per cent. On several other occasions irregular absorption behavior has been observed at 15° C. This is probably mere coincidence, however. The data are too meager to justify the suggestion that the irregularities may be due to a critical temperature relation. Individual peculiarities, including differences in absorptive capacity, will no doubt account for all the irregularities observed.

TABLE IV

RATE OF ABSORPTION IN CORN; TIME EXPRESSED IN MINUTES; RATE IN PERCENTAGE OF INTAKE PER MINUTE

PERCENTAGE INTAKE	TEMPERATURE											
	5°		15°		25°		35°		45°		55°	
	Time	Rate	Time	Rate	Time	Rate	Time	Rate	Time	Rate	Time	Rate
7.5..	98.06	0.02157	92.11	0.02835	55.82	0.05103	35.22	0.07866	26.55	0.11676	18.43	0.16674
10.0..	225.98	0.01777	184.65	0.02577	109.90	0.04201	70.30	0.06176	49.07	0.09781	34.75	0.14111
12.5..	381.60	0.01463	286.42	0.02343	175.59	0.03459	112.91	0.05332	77.75	0.08202	54.03	0.11943
15.0..	569.78	0.01206	398.41	0.02130	255.35	0.02848	164.68	0.04389	111.32	0.06863	76.81	0.10108
17.5..	798.52	0.00993	521.57	0.01936	352.22	0.02345	227.55	0.03614	151.18	0.05740	103.73	0.08554
20.0..	1076.25	0.00818	657.06	0.01760	470.02	0.01930	303.92	0.02975	198.75	0.04816	135.54	0.07239

TABLE V

TEMPERATURE COEFFICIENTS OF WATER ABSORPTION IN CORN

PERCENTAGE INTAKE	VELOCITY RATIOS AT GIVEN PERCENTAGE OF INTAKE				
	$\frac{\text{Velocity } 15^\circ}{\text{Velocity } 5^\circ}$	$\frac{\text{Velocity } 25^\circ}{\text{Velocity } 15^\circ}$	$\frac{\text{Velocity } 35^\circ}{\text{Velocity } 25^\circ}$	$\frac{\text{Velocity } 45^\circ}{\text{Velocity } 35^\circ}$	$\frac{\text{Velocity } 55^\circ}{\text{Velocity } 45^\circ}$
7.5....................	1.314	1.800	1.541	1.484	1.428
10.0....................	1.450	1.630	1.541	1.510	1.443
12.5....................	1.602	1.476	1.541	1.538	1.456
15.0....................	1.766	1.337	1.541	1.564	1.473
17.5....................	1.950	1.211	1.541	1.591	1.488
20.0....................	2.152	1.097	1.541	1.619	1.503
Average...........	1.706	1.425	1.541	1.551	1.465

Discussion

The quantitative study of absorption is beset with numerous difficulties, not the least of which is the variability of the absorbing material. The early intake data of table I show that corn grains are somewhat irregular in moisture intake during the first fifteen to thirty minutes, regardless of strict control of the conditions. After that time the intake is fairly uniform, barring internal physical changes. The behavior suggests that there is a certain amount of heterogeneity in the substance of the grains, which may bring about irregular absorption through variations in the quantity of the components present in individual seeds, the components themselves possibly having different absorption rates at the same temperature. After a time the intake becomes regular, and the temperature influence becomes visible. Our quantitative studies in this case deal only with the main curve, after the initial irregularities have disappeared.

So many cases of irregular absorption have been observed during the last ten years that one might be inclined to think that the cases which can be analyzed mathematically with any degree of exactness are the exception, not the rule. Sometimes the changes in the rate of absorption are so great that analysis becomes impossible, or meaningless, and would require tangent sectors of different types of curves to follow the data. Moreover, after such sectors had been worked out, it is possible that they could not be interpreted. The irregularities of intake result from a number of causes which may be briefly touched upon.

Heterogeneity is a rather common cause of irregular behavior, especially in seeds, where coats, embryos, and endosperm of different kinds may possess widely different physical and chemical constitution. If the seed coat is very porous, embryo moderately absorptive, and endosperm very hard and resistant, the absorption rate will be changed as each part absorbs its quota of water. In some cases impermeability of the coat may cause abnormal results. The hard seeds of legumes may be cited in this connection. In one experiment with beans, an individual seed lay for three days immersed in water before significant intake began. One such seed in an experimental group would cause the mathematician much trouble.

Another potent cause of irregularity was pointed out in the work on pea cotyledons. Internal breaks are of frequent occurrence. After running along uniformly for an hour or two, intake shows a sudden increase, the absorption for a given period being perhaps twice what one would expect from the preceding portion of the curve. In such cases, on careful inspection of corn grains one can often find cracks in the endosperm which are visible through the unbroken coat. The formation of the internal cavity sets up a partial vacuum, into which unabsorbed water is drawn. A number of carefully run series had to be discarded on this account. Accurate analysis would have been impossible.

Internal structure may modify absorption rates to such an extent that the data are not a measure of absorption as such. This is the case in dealing with absorption by small blocks of wood. Cubes of cherry wood not more than a few millimeters in diameter were kept immersed in water at 25° C., and, small as they were, it required from fifteen days to a month or more for them to reach their maximum weight. It is evident here that it is not simple absorption of water by wood that is being measured. The tracheae are full of air, and on immersion the water cannot enter and displace the air, but merely enters the open ends of the tracheae, and penetrates through the walls where there is no air to displace. The long, slow absorption period is probably dominated by the solution of the gas, as in waterlogging, and the data do not reveal the absorption rate of the wood substance. If the blocks are first strongly evacuated, and water introduced over them while in this condition, considerable water enters at once, and the whole curve of increasing weight is changed.

One of the most interesting cases of irregular absorption behavior was found in the tissue of *Auricularia*. Square pieces of the fungus were allowed to dry, giving pieces about 8 mm. square and 0.5 mm. thick. One of these pieces weighed 0.0242 gm. Immersed in water at 25° C., it required twelve days to reach its maximum weight, at which time the increase in weight was 2310 per cent. The rate was not uniform during the period, nor did the rate decrease in orderly fashion with time. In the first two minutes the intake was more than 100 per cent, mainly from the porosity of the outer layers of the tissue. During the first twenty-four hours the total absorption was 455 per cent, and in the second day it was 78 per cent. The rate of intake remained at about that level for a day or two, and then began increasing again. On the fifth day it was 129 per cent, the sixth day 160, and the seventh day 279 per cent. During this second rapid period of weight increase, the middle layer of the fungus tissue became clear, gelatinous, and highly swollen. Finally the piece split apart through the middle layer. In this case it seems highly probable that the rate of absorption is partially determined by chemical changes, perhaps in the nature of transformation and hydration of polysaccharide carbohydrates. After the tissue had passed the maximum point of increased weight at the end of twelve days, it was allowed to become air dry again. On reweighing, it was found to have lost 31 per cent of its weight during absorption. This behavior reminds one of the results obtained by MACDOUGAL, RICHARDS, and SPOEHR (10, 11), with other biocolloids, discs of cacti, etc.

These irregularities of behavior often make the problem of interpretation difficult, and one must expect specific behavior in all kinds of objects, depending upon structure, composition, physical and chemical behavior of the materials used. It follows, also, that there is probably no rate law for absorption. The fact that a single type of curve has been successfully fitted to the absorption data of several types of seeds is not sufficient evidence for a rate law, but merely shows that when conditions are uniform inside and outside the absorbing body, the processes of water absorption proceed regularly, in accordance with the physical and chemical forces operating.

One of the problems connected with the investigation of absorption rates, particularly in the seeds of the Gramineae, is the localization of water intake by the investing membranes of the caryopsis. COLLINS (7) studied absorption by barley grains, and came to the conclusion that there are localized areas at the germ end of the grain through which most of the water passes. Using iodine solutions to render the progress of intake visible, he drew the general conclusion that "the entry of iodine solutions into the barley grain is not general and uniform over the whole surface of the grain, but its uptake is localized at the germinal end, and it is distributed peripherally in the endosperm along the subaleuronic layer of starch cells of the curved surface." Tests with acids and stains seemed to confirm the idea that both water and solutes gain entry in the micropylar region through a selectively active tract of cells which constitute a specialized local path of entry.

The same general conclusion was reached by HARRINGTON and CROCKER (9), who studied the entry of iodine solutions into the caryopses of Johnson grass and Sudan grass. The solution seemed to enter through the hilar orifice, or micropyle, or both, and to spread in a distal direction along the inner surfaces of the aleurone layer and scutellum.

Preliminary studies made on corn at the University of Kansas indicated general permeability of corn seed coats to water. LOVEJOY'S osmosis tests were made with coats taken from the main lateral areas of the grain; and these were not only permeable to water, but also allowed salts used as solutes to pass out slowly during the exhibition of osmotic action. These facts point to coat permeability to both water and salts. Some iodine tests made at the University of Kentucky indicated that the coats of corn allow iodine also to penetrate the surface of the grain generally, a fact observed by HARRINGTON and CROCKER. The entry in the case of Hickory King corn, however, appears to be more rapid over the flank surfaces than over the distal areas of the coats. Water also enters with considerably greater rapidity through the proximal portions of the integuments, even when the micropylar and hilar areas are sealed over. These iodine studies (1) indicate that COLLINS, and HARRINGTON and CROCKER have possibly misinterpreted the progressive distalward staining of subaleuronic tissues with iodine. The evidence is clear in the case of corn that there is very little transfer of iodine through the tissues in any direction, but that the iodine is adsorbed or chemically combined as it enters, and is not very free to travel through the tissues. The appearance of distalward movement in barley is undoubtedly due to differential permeability of the caryopsis integuments, the iodine penetrating more rapidly over the proximal regions, and progressively less rapidly toward the distal end of the grain. This gives, with time, the appearance of a distalward movement of iodine in the subaleurone region, which in reality does not take place. This situation has been demonstrated for wheat by BRAUN (2) at the Cincinnati meeting of the Botanical Society of America. His work and the work done in the Hull Botanical Laboratories on this point are in general agreement.

Since REICHARD (13) had associated the semipermeability of barley seeds with a tannin layer, some microchemical tests of corn were made, on both yellow and white corn seed coats. The tests showed only minute amounts of tannin present in isolated cells or groups of cells. No evidence of a tannin layer was found, so that any semipermeable properties present do not depend upon tannins. In this connection it should be noted that COLLINS was not able to confirm REICHARD'S claim of a definite tannin layer in the barley grain.

The main purpose of this work was to put the chemical theory of water absorption proposed by BROWN and WORLEY (6) to a crucial test. If absorption is determined as to rate by chemical simplification changes in water, the polyhydrones dissociating as the temperature rises and providing larger quantities of simple hydrone which alone can pass the membranes, and if it has a temperature coefficient of two or above, then a rise of 50° C. should result in a rate of intake about thirty-two times as great at the highest temperature as at the lowest of the range used. If the increase were not so large, the amount by which it fell short would be a general measure of the failure of the theory.

The total increase in absorption rate for the 50° rise can be obtained from the ratio 50°/5°, or the five average ratios of table V may be multiplied into one another successively. By either method it is found that the rate of water intake at 50° C. is somewhat more than eight times as rapid as at 5° C., instead of thirty-two times as great. In other words, the increase in absorption brought by a 50° rise in temperature is only about one-fourth as much as would be expected if the process were determined by some chemical change, as has been proposed. The results are strongly against the chemical hypothesis.

Attention is also called to the fact that the values of Q_{10} in table V show little if any tendency to decrease at high temperatures, as one might expect from the Arrhenius temperature law, if we are dealing with a chemical process. While the formula upon which the Van't Hoff rule is based does not provide for a smaller temperature coefficient as the temperature rises, the more correct formula of the Arrhenius law shows from its plotted curve (12) that the value of Q_{10} tends to decrease at high temperatures. Biological processes involving chemical changes frequently show reduction of the temperature coefficient as the temperature becomes extreme. In some cases these results are caused by the harmful action of heat upon the protoplasm; but such reductions at high temperatures are in agreement with the Arrhenius law, and would be expected even if the protoplasm were to remain unharmed by the heating. While our results are made irregular by a poor 15° curve, the average value of Q_{10} at the highest temperature used is still higher than the values at 25° and 35°, and only slightly below that at 45°. There is no striking tendency to decrease as shown in these figures, and very little should be expected if we are dealing with a process largely non-chemical in nature.

The data as a whole constitute additional evidence in favor of the point of view developed in previous work, that absorption is as complex as many other life processes, and involves both physical and chemical factors. The fact that the value of Q_{10} is not so very much above the coefficient for physical processes should indicate that it is mainly a physical process, as has always been assumed. Furthermore, it seems very probable that temperature effects upon the seed colloids account satisfactorily for the slightly higher value of the temperature coefficient for absorption in corn.

Since it has been possible to run these curves very close to the experimental data with the same logarithmic formula as was used with *Xanthium* seeds and split peas, it is clear that the data are adverse to the idea that the velocity of absorption is an exponential function of the temperature. Whenever it is possible to apply the equation used in this paper to the curve of absorption, the velocity is determined by the previous absorption, rather than by temperature. The formula which expresses this relation has been given in the introductory paragraph. The corn absorption is then another case in which the velocity of intake at any moment is an inverse exponential function of the total previous intake. In discussing the problems of permeability, STILES (18) remarks that, aside from the precision in measuring tangents, and from them the velocity of absorption, such formulas as the equations used to represent the curves of water intake in this work do not tell us much about the nature of absorption and permeability. This critical judgment of the method is well taken; and the only use we originally had for the formulas was to substitute precise methods for rough methods of measuring tangents. For that purpose any regular mathematical expression that followed the data faithfully could have been used with equally precise results. It would be hard to find a mathematical formula that serves this purpose any better than the one here used. The only other conclusion that we reach from the mathematical expression used is the conclusion as to the velocity being an inverse exponential function of the previous absorption; and that conclusion is implicit in the formula used, a conclusion made necessary by the nature of the equation. The conclusion that the process of absorption is physical mainly rests on the Q_{10} values, which would have been the same if calculated from any other curve following the same data.

In BROWN and WORLEY'S theory of water intake, the semipermeable membrane had an important rôle in determining the rate of water penetration. It was held that only simple hydrones could pass through such a membrane, while the complex polyhydrones or associated H_2O groups would be held back. Water absorption through semipermeable seed coats, however, has about the same temperature coefficient as absorption in the absence of a membrane. This makes it improbable, as was previously stated, that semipermeability is important in determining the rate of absorption of water, *when supplied as distilled water.*

During the last twenty years the fact that many seeds are provided with selectively permeable coats has become well established in the literature (3, 4, 8, 15, 16). An occasional paper appears, however, in which the significance of this fact seems to be overlooked, and it is advisable to lay emphasis upon the presence in seeds of osmotic membranes, capable of showing the same behavior toward solutions as do membranes of living protoplasm.

In all cases where such membranes are present, they will affect the rate of water intake if water is supplied in the form of a *solution*, whether of nutrient or non-nutrient salts.

This seed coat condition, for instance, accounts for a large part of RUDOLFS' (14) results, although he mentions none of the pertinent literature. The behavior which he describes is exactly what would be expected of seeds possessing semipermeable coats, and has exactly the same significance as the fact that a saturation deficit can be produced in a living cell possessing a semipermeable protoplasmic membrane, by means of KNO_3 solutions. Of nine kinds of seeds used by RUDOLFS, six are from families in which semipermeable coats have been described. The mere fact that nutrient salts were used does not change the nature of the phenomenon. In the cases where absorption seems to be increased above normal by salt action, the formation of soluble organic materials within the semipermeable seed coat may account for the results, or we may be dealing with membrane equilibria. In *Xanthium* seeds the formation of osmotically active solutes inside the seed leads to excessive water intake. This is particularly true with KOH and acetic acid. Possibly the behavior of K_2CO_3 in RUDOLFS' work is to be explained mainly on the basis of the hydrolysis of the salt, the production of KOH, and the internal effects of KOH on the seed substance, coupled with semipermeable coat effects. This problem has received critical consideration by BROWN and TINKER (5) with reference to phenols and acetic acid.

The work presented here confirms in detail the results obtained several years ago with seeds belonging to families far removed from the Gramineae. The mere fact that seeds of *Xanthium* are fatty, peas rather high in protein, and corn rich in carbohydrates, has little to do with the physics of absorption. It may have something to do with the total amount taken in at saturation, for corn saturates at more than 60 per cent, *Xanthium* at a little over 50 per cent, peas at 75–90 per cent (according to the variety), and some other legumes at still larger amounts; but the velocity apparently is determined by physical factors largely, with the state of the colloids modifying to a certain extent the rate of water entry.

Summary

1. A study of the influence of wide temperature differences on the rate of water absorption by seeds of corn has been made, with results in complete agreement with a similar study of *Xanthium* seeds and pea cotyledons made previously.

2. The same type of formula, and the methods of mathematical analysis used with other seeds, were found to apply with great exactness to the data obtained with corn.

3. The temperature coefficient over the range from 5°–50° C. averages 1.537, somewhat above the coefficient for purely physical processes. This coefficient is to be compared with that of 1.55 for *Xanthium* seeds, and 1.6 for pea cotyledons.

4. The rate of absorption at 50° C. is somewhat more than eight times as fast as at 5° C., whereas the chemical theory of absorption would call for a rate thirty-two times as great.

5. The increase is therefore only one-fourth of what one would expect if absorption rates were determined by hydrone simplification of water as the temperature rises.

6. From mathematical considerations, the velocity of intake at any given moment must be considered as approximately an inverse exponential function of the amount of water previously absorbed.

7. Attention is called to a number of cases of irregular absorption rates, which indicate that many substances have specific absorption behavior. A rate law with wide applicability is not to be expected.

8. The importance of semipermeability with reference to the intake of water from solutions of various kinds is pointed out.

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EMBRYOGENY OF ABIES

A. H. HUTCHINSON

(WITH PLATES XVII–XX AND THREE FIGURES)

This investigation of the embryo development in *Abies* was begun during the summer of 1914. Since that time much additional material has been collected, in order that gaps might be filled in the series and that no stages might be omitted. The most complete series is that of *Abies balsamea* collected in Algonquin Park, Ontario, but sufficient sections of *A. grandis* and *A. amabalis* have been made to establish the similarity of these species with respect to the development of their embryos. MIYAKE (8) studied the early stages of the proembryo of *A. balsamea*, and his figs. 35–40 show that the development up to the eight-celled stage is similar to that of *Pinus*. BUCHHOLZ (1, 2) states that *Abies* "probably has proembryos identical with *Pinus*," and that "in a few rare instances a divided rosette cell and a more advanced rosette embryo were found; cleavage polyembryony was found in a few cases." Beyond this stage no records are available.

Proembryo

In an earlier paper (6) the first division of the zygote nucleus has been described. The first and second free nuclear divisions occur during the time the proembryo is moving from the central area of the egg cytoplasm to the pole remote from the micropyle. During the late telophases and until the early prophases the nuclei are quite large, 40–50 μ in diameter, and show vacuolated chromatic strands (text figs. 1–3). At the time of fertilization the egg cytoplasm is filled with large and abundant food bodies, chiefly starch grains. This food is rapidly digested and utilized to such an extent that when the four nuclei have reached the abmicropylar pole, the cytoplasm, which has accumulated in the same region, contains only a small residuum of finely granular food material surrounding the nuclei. The next divisions result in two tiers of four cells each, the abmicropylar cells containing the greater portion of the remaining food store, while the nuclei of the open cells have a very limited supply (figs. 3-6). It seems probable that this condition may be a determining factor in the modification of succeeding stages of development.

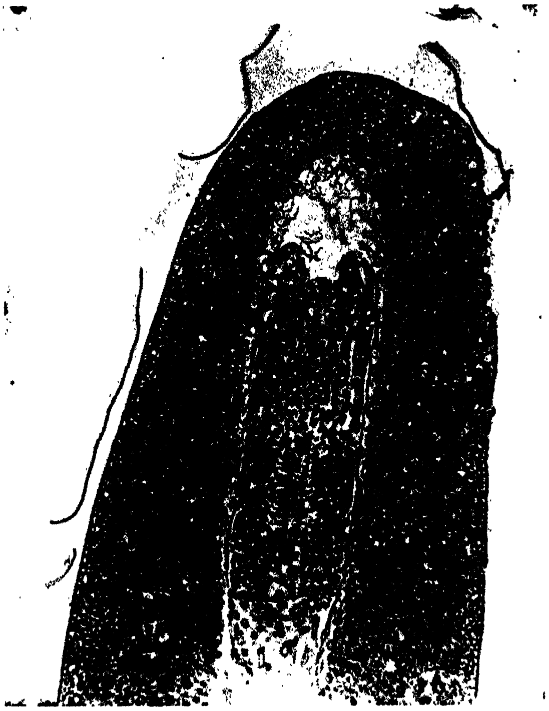

FIG. 1.—Embryo of *Abies* surrounded by gametophyte

Ordinarily the proembryo development ends at the eight-celled stage, the four abmicropylar cells being primary embryo cells and the admicropylar four resembling the open tier of *Pinus* (figs. 3, 5, 6, 7). Out of many, only three cases were found where a third tier was interposed, and in these instances the cells were scantily supplied with cytoplasm, platelike in form, and suspended in the open region resulting from the rapid movement of the primary embryo cells toward the abmicropylar gametophyte tissue. Whether this interposed tier is homologous to the suspensor cells or to the rosette cells can only be conjectured, largely because of their infrequent occurrence and their temporary nature. Moreover, no divisions have been found in the open tier of cells, and since the movement of the abmicropylar cells begins very soon after their formation, it is difficult to determine whether the division of the tier in any given case is about to result in the production of primary embryo cells and suspensor cells, or of the cells of the embryo and embryonal tubes. It is remarkable that, with few exceptions, neither rosette nor suspensor cells are formed. The nuclei of the open tier rapidly disintegrate, while the primary embryo cells move into the gametophyte tissue and continue digestive and mitotic activity.

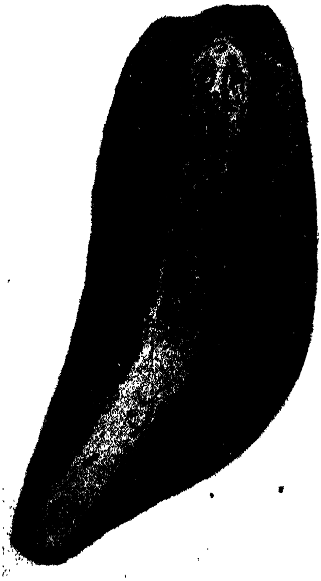

FIG. 2.—Embryo of *Abies*, showing early stage in development of cotyledons.

Cleavage polyembryony

Cleavage polyembryony occurs in approximately 10 per cent of the cases studied (figs. 8, 9, 11). Not infrequently as many as four embryos reach the eight to sixteen-celled stage before elimination through competition begins (fig. 8). Cleavage of the primary embryo cells depends directly upon the completeness of the fiber system formed during the eight-celled stage. In some instances the fiber area reaches to the tip of the proembryo (fig. 5), but more frequently it forms an incomplete plate between the primary embryo cells (fig. 3), and in some cases this plate seems to be absent. Where complete cleavage does not take place there may be a variable degree of separation at the admicropylar end of these cells (figs. 6, 7). The wall does not form in the ordinary way along the central axis of the fiber mass, but instead a membrane is laid down at the margins of the fiber area, and as the fibers disintegrate the delimited area remains as an open intercellular space. In the reduced proembryo of eight cells the fibers between the two tiers of cells have a similar history. In this manner the primary embryo cells become free to move into the gametophyte tissue (figs. 3, 5, 6, 7). There is evidence that cleavage polyembryony in *Abies* is the result of this peculiar development of detached pairs of adjacent cell walls during the mitoses of the proembryo.

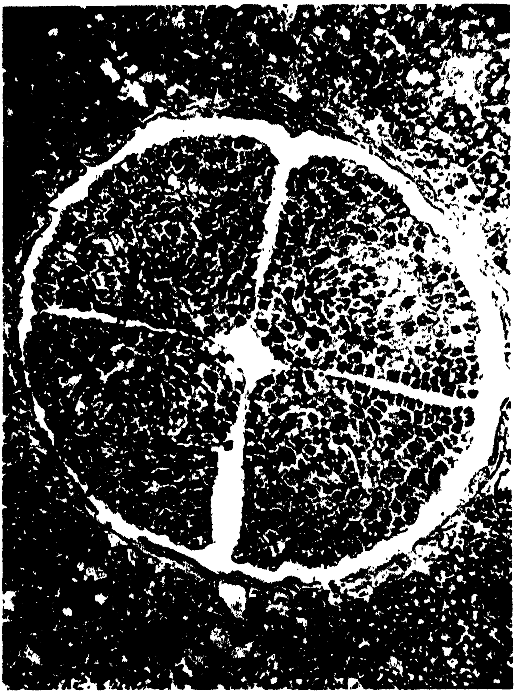

FIG. 3.—Transverse section of cotyledons of *Abies*

Intercalary growth of embryo

The early growth of the embryo is linear. The division of each primary embryo cell is followed by a number of intercalary divisions in the same direction. In embryos where cleavage has been initiated in the proembryo the result is a filament of six or eight cells (fig. 9), several of which have elongated to form embryonal tubes, otherwise there are four (or sometimes two) chains of cells joined to form a single embryo, each chain elongating by intercalary divisions (figs. 8–14). Fig. 13 shows a case where an admicropylar cell of one series has divided first, while the abmicropylar cell of the other series has been first to divide. The only cells which do not divide are some of the admicropylar which develop into embryonal tubes. It is during this period of intercalary growth that the embryo moves most rapidly in the abmicropylar direction.

Formation of massive embryo

Linear, intercalary growth with the formation of transverse walls is followed by divisions, the walls of which are in the direction of the main axis (figs. 9, 11, 14, 16). The filamentous embryo, resulting from cleavage of the proembryo, is thereby converted into an embryo of four series of cells, which resembles the preceding stage of an embryo where no cleavage has taken place (fig. 11). Periclinal walls are formed next, producing central and peripheral areas (figs. 15, 17). These regions are the primary structural units, and their identity is maintained throughout the development of the embryo. The peripheral layer, or protoderm of HABERLANDT (5), is the first region to become differentiated. The cells of this layer covering the abmicropylar end of the embryo divide only by anticlinal walls, giving origin to the epidermis; whereas the cells at the admicropylar end divide in all planes but most rapidly in the plane of the longitudinal axis, thereby giving origin to the massive structure which at first is suspensor-like in function, and which later becomes the coleorhiza and root cap, successively (fig. 18). The cells of the central area divide at a fairly uniform rate in all planes, thereby producing a globular mass of undifferentiated cells (fig. 18). The admicropylar elongation at this stage is accompanied by an abmicropylar movement of the embryo and by a rapid disintegration of the terminal cells. The embryo at this time is decidedly club-shaped.

Mitotic activity

The primary differentiation of the embryo may be expressed almost entirely in terms of mitotic activity. As already noted, the protoderm is the first region where the planes of division are limited in number, and the regularity of these divisions gives rise to the epidermis and coleorhiza. The cotyledons are the result of decreased or suppressed mitotic activity at the abmicropylar end of the longitudinal axis, accompanied by constant or increased activity at the marginal region surrounding this area. The nuclei of the former region are in the resting condition, as shown by their many nucleoli, whereas the commonly occurring mitotic figures in the latter region give evidence of both dermal and subdermal activity. Inequalities in this activity produce lobes or the primordia of cotyledons (figs. 19, 20). The number of these areas of increased mitotic activity and hence the number of cotyledons varies about the mean of four or five. At this time the cells of the embryo are all nearly isometric, and there is no apparent differentiation of the cytoplasm. Another area of suppressed mitotic activity is found at the admicropylar end of the central area in contact with the developing root cap (fig. 21). This group of cells is similar in position and general conformation to those cells which many have called the "initials." These have shown repeatedly, however, the same resting characteristics as the dormant stem tip cells, whereas the cells surrounding them are mitotically active. When the cotyledons have attained approximately one-half their mature length the procambial strands begin to appear. In some instances they may be detected in the admicropylar or radicle region first, but ordinarily their appearances are simultaneous from the margin of the region of suppressed activity at the tip of the radicle to the terminal region of the cotyledons. The procambial strands are composed of cells which resemble the cambium in mode of division, that is, their walls are in the direction of the longitudinal axis only. The tension resulting from transverse divisions in the adjacent cells causes the elongation of the procambial cells.

In following the development of primary morphological units in the embryo of *Abies*, one is forced to the conclusion that these units are not functional primarily, but are the result of the nature and degree of mitotic activity, and later they perform some function or other. The epidermis arises as a peripheral region of anticlinal walls and becomes a single-layered, absorptive and later a protective region. The root cap arises as a peripheral region of periclinal divisions chiefly, and becomes a suspensor and later a protective region. The cotyledons arise as regions of augmented mitoses and become food storage organs and later a source of food. The stem tip arises as a region of suppressed mitotic activity and later becomes the most marked of growing regions. The procambial strands originate as regions of axial divisions, and by similar divisions continue to give rise to conductive cells. The little explored field of investigation which deals with the factors determining the nature and rate of mitosis is basic to any explanation of embryo development.

Discussion

The events of the very early proembryo as described in this paper confirm the account of MIYAKE (8). There is, however, a very marked difference between the later embryos found in my specimens of *Abies* and those described by BUCHHOLZ (2, 4). The latter states that "in *Abies* the normal product of a fertilized egg is a single embryo. The group of rosette cells is present, and in a few rare instances a divided rosette cell and a more advanced rosette embryo were found." On the contrary, cleavage polyembryony frequently occurs in *Abies*, a distinct separation being evident in 10 per cent of the instances and an incomplete cleavage in a large proportion of those remaining. No rosette has been found, but vestiges of what may be regarded as rosette cells were seen (fig. 4), and it is not probable that these would have been seen in the dissected embryos studied by BUCHHOLZ. The fact that an embryo sometimes begins to develop from the fertilized ventral canal cell (6), together with the difficulty which BUCHHOLZ found in clearly distinguishing the cells which he describes as rosettes, may explain the apparent contradictions. To quote: "The basal plate, a deposit formed within the egg over the rosette cells, is very thick and frequently obstructs a clear view of the rosette cells, which also collapse early unless a rosette embryo happens to develop." The embryos of *Abies* which I have examined resemble those described for *Pseudotsuga* (4) more nearly than those of any other conifer.

The peculiar mitotic figure described by Miss KILDAHL (7) for *Pinus* in the proembryo is similar to those occurring in *Abies*. In *Pinus* the plate of the unusual fibers is axial in direction, and may be regarded as accounting for the cleavage polyembryony, as in *Abies*. In *Abies* similar structures with resulting walls are formed transversely, thereby making possible the movement of the primary embryo cells away from the open tier.

The embryo of *Abies* shows many advanced characters, chiefly in the form of reduction and the elimination of ancestral or primitive features, notably the rosette cells, suspensor cells, and the apical cell. Another feature which may or may not be advanced is the occurrence of cleavage polyembryony. BUCHHOLZ regards this as a primitive character. Primitive characters are usually constant features of lower orders or even of divisions, such as is the case with suspensors and apical cells, or are present in geologically more ancient forms, but on a basis of such evidence there is no reason to suppose that cleavage polyembryony is primitive. Moreover, *Abies* furnishes rather satisfactory evidence that cleavage polyembryony is a derived condition. Ordinary cell divisions in the proembryo result in single embryos, while modified divisions (figs. 5–7) result in cleavage of the proembryo. It may be noted also that the proembryos which are most reduced in the number of cells show cleavage, while those which are less reduced (fig. 4) do not show cleavage. To concede that cleavage polyembryony is a derived character does not affect the position of *Pinus* necessarily, since it has very frequently been demonstrated that living forms of remote lineage exhibit a complex of derived and of primitive characters. In any event, it may be conceded that the proembryo and early embryo of *Abies* are advanced or derived in many respects.

Summary

1. The proembryo of *Abies* ordinarily consists of eight cells only, in two tiers, the admicropylar open tier and the abmicropylar primary embryo tier of cells.

2. Very infrequently a third tier is interposed, which may represent the rosette or the suspensor.

3. Cleavage polyembryony frequently occurs, and is the result of modified spindle structures.

4. The early embryo grows by intercalary divisions; an apical cell is not formed.

5. Primary periclinal divisions delimit the central region from the protoderm; these regions persist as primary structural units.

6. Regions which become tissues are differentiated primarily because of specific mitotic characters and later assume functional characters.

7. The stem tip and the "initials" are regions composed of resting cells in the early embryo.

8. The embryo of *Abies* shows many advanced or derived characters.

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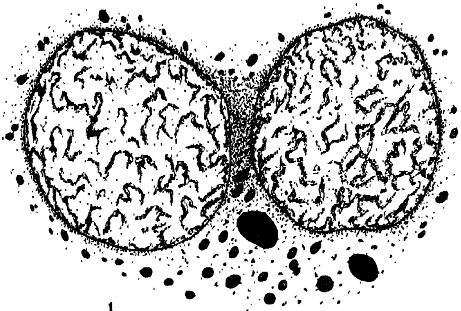
DESCRIPTION OF PLATES XVII–XX

All figures are drawn with the aid of a camera lucida.

FIG. 1.—Nuclei resulting from first division of zygote, imbedded in egg cytoplasm.

FIG. 2.—Two of the four nuclei which have migrated to abmicropylar end of egg cytoplasm.



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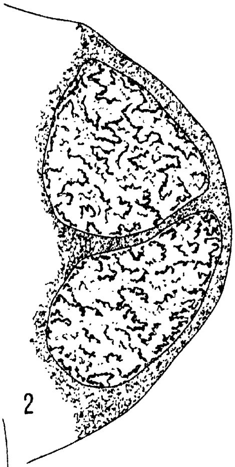

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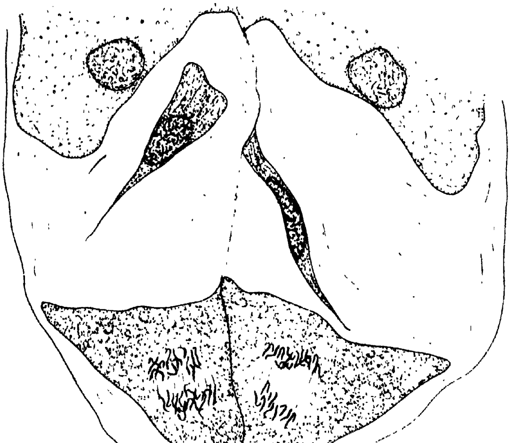

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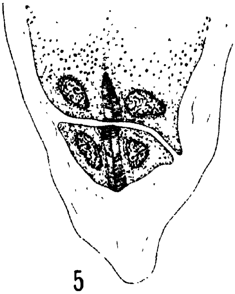

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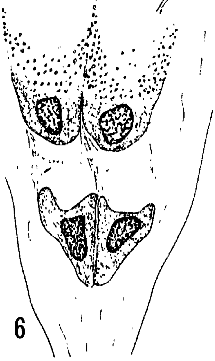

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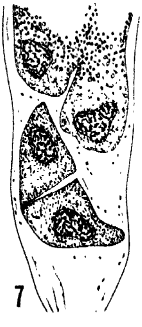

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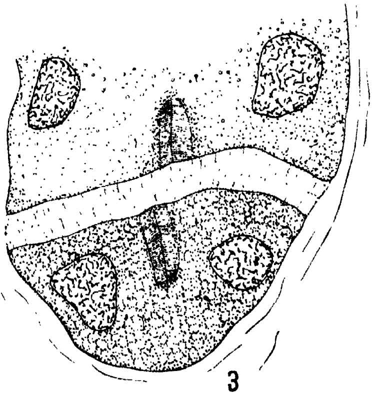

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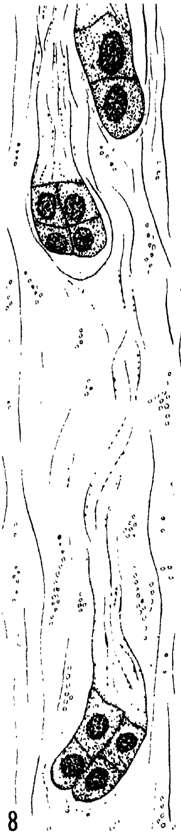
HUTCHINSON on ABIES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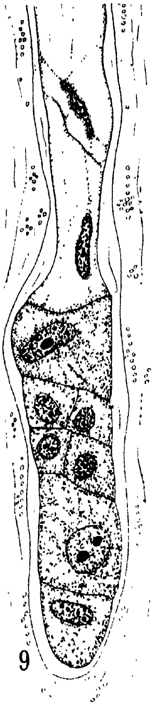
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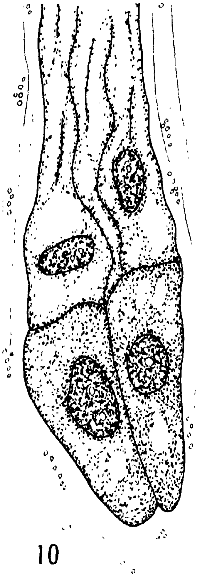
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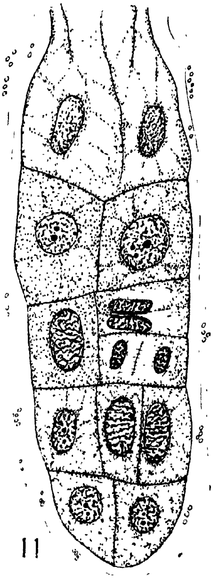
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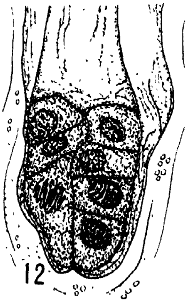
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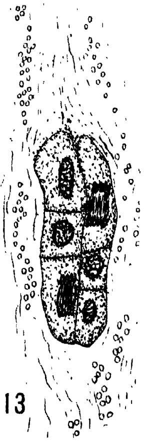
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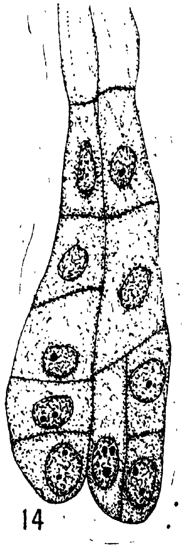
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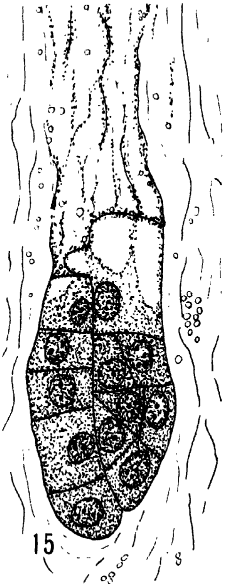
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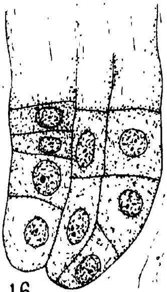
HUTCHINSON on ABIES



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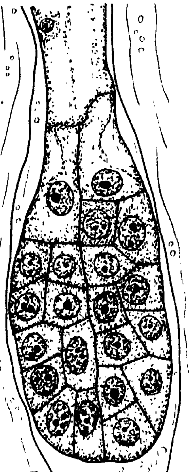

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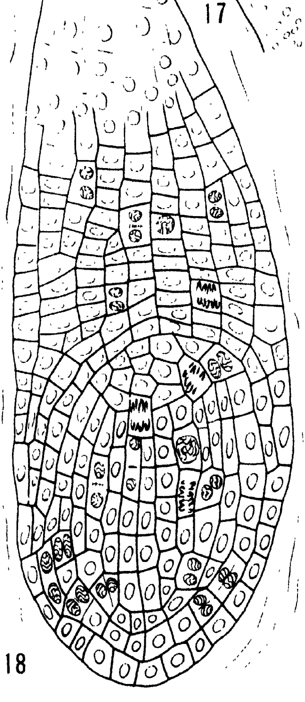

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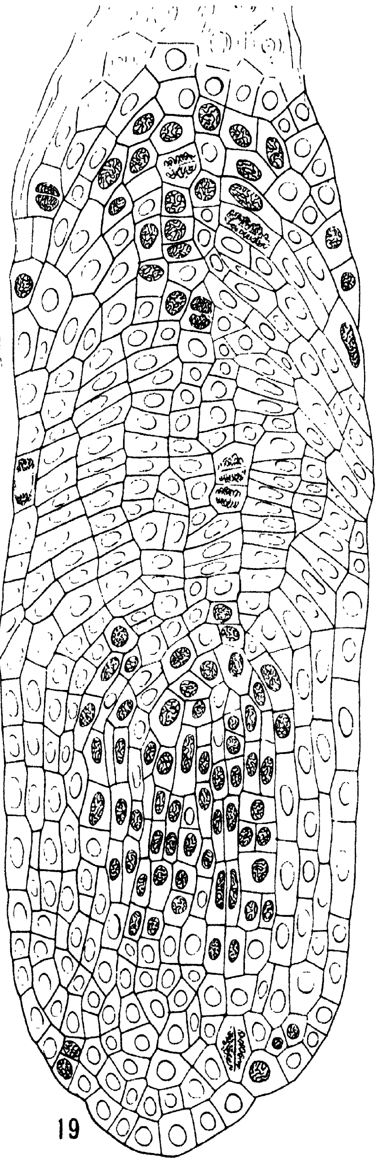

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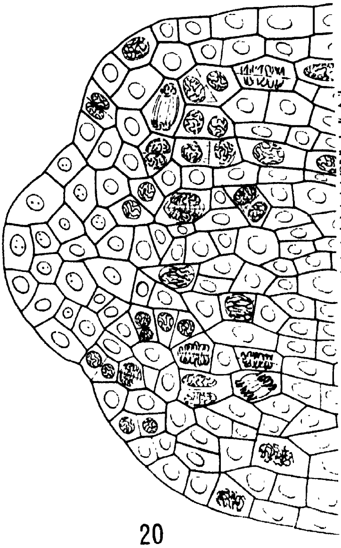
HUTCHINSON on ABIES

PLATE XX



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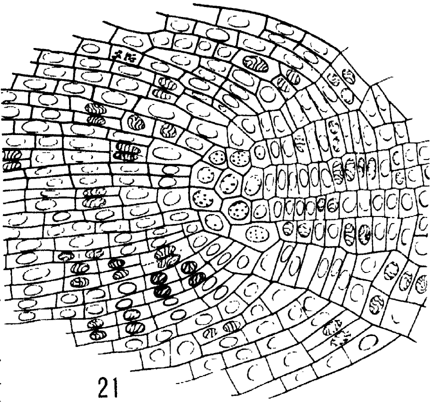

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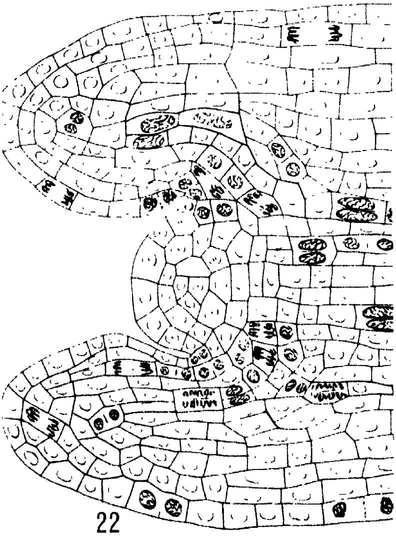

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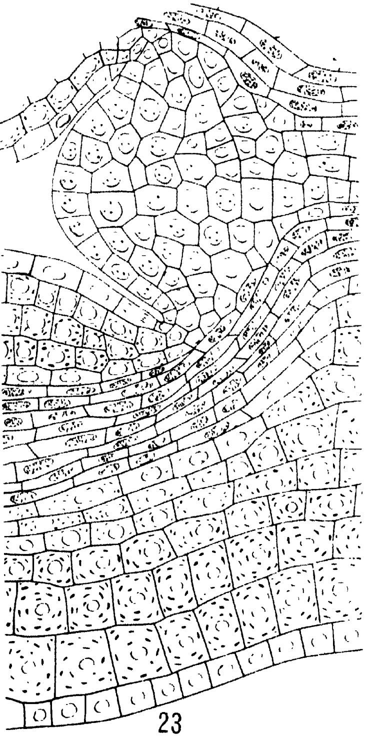

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HUTCHINSON on ABIES

FIG. 3.—Eight-celled stage which proves to be complete proembryo in most instances, showing primary embryo cells and open tier; note transverse plate of disintegrating fibers and two parallel walls separated by fiber area, also axial fiber plates, incomplete.

FIG. 4.—Unusual embryo, showing vestigial cells interposed between open tier and primary embryo cells; latter have moved some distance in abmicropylar direction and are dividing to form terminal embryo cells and embryonal tubes.

FIG. 5.—Proembryo in which transverse plate of fibers has disintegrated, releasing primary embryonal cells; axial plate is complete as in cases of cleavage polyembryony.

FIGS. 6, 7.—Primary embryo cells moving away from open tiers.

FIG. 8.—Three of four embryos which have resulted from cleavage of proembryo, surrounded by food material derived from gametophyte; embryonal tube cells rapidly disintegrating; axial divisions already taken place in two embryos.

FIG. 9.—Embryo which has resulted from cleavage of proembryo and which formed filament of eight or more cells before axial walls were formed.

FIGS. 10, 12, 13.—Embryos composed of four series of cells.

FIGS. 11, 14, 15, 16.—Appearance of periclinal walls.

FIG. 17.—Primary periclinal walls complete, thereby delimiting primary central and peripheral regions.

FIG. 18.—Massive embryo; peripheral region (protoderm) showing two areas, abmicropylar with anticlinal divisions only, admicropylar with periclinal divisions chiefly; central region undifferentiated.

FIG. 19.—Massive embryo, similar to fig. 18, except that increased mitotic activity is giving rise to cotyledonary protuberances.

FIG. 20.—Increased mitotic activity in region of cotyledonary outgrowths associated with resting condition of cells at abmicropylar tip.

FIG. 21.—Origin of procambial strands in root as cells dividing only by axial walls; "initials" in resting condition, their nuclei having numerous nucleoli.

FIG. 22.—Later stage of cotyledons with procambial strands appearing; stem tip in resting condition.

FIG. 23.—Differentiation of stem tip, procambial strands, cortex, and epidermis.

IRON SUPPLY IN A NUTRIENT MEDIUM[1]

H. S. REED AND A. R. C. HAAS

It has long been known that most plants become chlorotic when the nutrient medium is deficient in soluble iron salts. The problem of a suitable iron supply, although of great importance in artificial cultures, has never been solved satisfactorily. The composition of many nutrient solutions is such that soluble salts of iron cannot remain in solution for more than a brief period of time. Investigators have sought to meet the problem by frequent renewals of the solution, or by frequent additions of iron salts. The earlier workers generally employed inorganic salts of iron, but recent workers have also employed organic combinations, such as the citrate or tartrate. The "soluble ferric phosphate" seems to be a very satisfactory form of iron for water cultures. DUGGAR (3) pointed out that this material seems to give a colloidal solution of high dispersity and high stability, even in the presence of other salts. The possible significance of the colloidal state of nutrient salts for plants has recently been suggested by COMBER (1).

In the course of an investigation on the growth of citrus trees and their absorption of materials from nutrient solutions, numerous cases of chlorosis appeared from time to time, despite the fact that special attention was given to the supply of iron salts. Not all cases of chlorosis in the trees could be correlated with an insufficient supply of iron. They often appeared to be connected with nutritional disturbances due to the absence of other nutrient ions. For example, when orange trees were grown in sand cultures receiving a potassium-free solution, incipient chlorosis appeared in the young leaves, but later the normal green color developed. Trees grown in sand cultures receiving a magnesium-free solution developed another type of chlorosis, which spread from a narrow strip along the midrib of the leaves until a considerable portion of the area was affected. GARNER (4) has shown that certain types of chlorosis in tobacco may be due to a deficiency of magnesium or of potassium. MILLER (11) and others have observed chlorosis of lemon leaves, which was due to a peculiar physiological condition of the trees, caused by the growth of adventitious roots. When lemons have been budded on sour stocks it sometimes happens that roots will grow from the base of the lemon trunk, just above the bud union. In such cases the branches directly above these adventitious roots usually bear chlorotic leaves. Recovery of these leaves follows the amputation of the root. The relation between the adventitious roots and the chlorotic leaves is not understood.

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[1] Paper no. 104, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, Cal.

The relation of iron to chlorosis in citrus trees is not so certain as one might conclude from a hasty survey of the problem, although there are many cases in which the lack of iron is unquestionably the causal factor. Chlorosis may appear in citrus trees in the field, when the roots come in contact with a large excess of calcium and magnesium carbonates, or a large excess of sodium and potassium carbonates (LIPMAN 9).

> Whether or not the chlorosis on the two types of abnormal soils is identical in nature, it is difficult to say with finality. There are certain differences in the appearance of trees and foliage between the two cases which counsel caution in rendering a decision on the point in question, and yet, in general, the disease seems to be the same.

From analytical data of soil extracts from normal and corral soils, LIPMAN reports that the extract of the normal soil was higher than that of the corral soil in total solids, volatile solids, nonvolatile solids, nitrates, calcium, and magnesium, but that the reverse was true for phosphorus, potassium, and sodium. The OH-ion concentration of the corral soil was high, and no soluble iron was found below the first 8 inches of soil in either case. LIPMAN is of the opinion that a lack of usable iron for the normal functioning of the chlorophyll in citrus leaves still seems the best explanation for the cause of citrus chlorosis, although certain aspects of the question are obscure.

GILE and CARRERO (7) have shown that for rice plants neither increased calcium assimilation nor mere alkalinity is the primary cause of chlorosis, but that it is the insolubility of iron brought about by these secondary factors. GILE (5) analyzed pineapple plants grown in soils high and low in calcium carbonate, and found that the chlorotic plants on calcareous soils contained more calcium and less iron in the ash, the other differences being slight or inconstant. He also found that the alkalinity induced by increasing amounts of sodium carbonate greatly depressed the growth without affecting the color of the plants.

McCALL and HAAG (10) have reported chlorosis in wheat plants in sand cultures when grown at different P_H values ranging from P_H 4.02 to P_H 7.0. VAN ALSTINE (14) found slight chlorosis in plants grown in solutions having a P_H value of 4.1. HOAGLAND (8) has emphasized the necessity of soluble iron in culture solutions, and has pointed out that the presence of sufficient dissolved iron in the culture solution will depend upon the form and quantity of the iron salt used, upon the concentration and reaction of the solution, and upon the length of time of standing. TOTTINGHAM and RANKIN (13) have shown that ferric citrate is a better source of iron for wheat seedlings than ferric sulphate, ferrous sulphate, or ferric phosphate, and possesses a fair degree of solubility over a considerable range of P_H value of the nutrient solution. The variation in efficiency of iron in the different forms supplied is correlated with variation either in the solubility of this element or in the modification of the P_H value of the nutrient solution.

It was with a view to determining some of the conditions which affect the amount of soluble iron salts in nutrient solutions that the following studies were undertaken. The problem is one of considerable importance for the intelligent use of nutrient solutions.

Effect of reaction upon amount of iron in solution

The solution designated as Hoagland's nutrient solution has been successfully employed in the experimental cultures of citrus trees and other plants. It contains 1455.1 parts per million of salts. Its composition is shown in table I in the column designated "1-5." The reaction of the freshly prepared solution was P_H 5.2. The other solutions whose compositions are given in table I are modifications of Hoagland's solution.

Tests were made to determine what changes in the solubility of iron salts occur in two types of nutrient solution used in experiments with young citrus trees. A set of ten flasks, each holding one liter of Hoagland's nutrient solution, was prepared in such a way that they contained from 5 to 25 parts per million Fe (table II). Five of the flasks were given 300 gm. of pure silica sand and shaken.

TABLE I

COMPOSITION OF NUTRIENT SOLUTIONS

Series	1–5 (p.p.m.)	30–35 (p.p.m.)	36–41 (p.p.m.)
Fe	1	1	1
Mn	0.1	0.1	0.1
Ca	159	....	159
Mg	54	54	54
K	185	406	406
Na	7	280	280
PO_4	105	105	105
SO_4	216	214	214
Cl	10	10	291
HCO_3	....	725	725
NO_3	718	718	718
Total concentration	1455.1	2603.1	3043.1

TABLE II

CHANGES IN SOLUBILITY OF IRON SALTS IN CULTURE SOLUTIONS

Culture solution	Number of days before making tests	Parts per million of Fe supplied				
		5	10	15	20	25
		Parts per million of Fe found				
Hoagland's	1	0.5	1.0	1.5	1.7	2.0
Hoagland's	8	0.4	0.5	0.8	1.1	1.3
Hoagland's plus sand	1	0.5	1.0	1.5	1.7	2.0
Hoagland's plus sand	8	0.1	0.1	0.1	0.2	0.5
Hoagland's without Ca, plus $NaHCO_3$	2	0.2	0.3	0.5	0.8	1.0
Hoagland's without Ca, plus $NaHCO_3$	28	0.2	0.2	0.2	0.2	0.2
Hoagland's without Ca, plus $NaHCO_3$ plus sand	2	0.2	0.3	0.5	0.8	1.0
Hoagland's without Ca, plus $NaHCO_3$ plus sand	28	0.2	0.2	0.2	0.2	0.2

Another set of ten flasks was prepared in the same way as the first except that the solution was modified by the addition of 1000 parts per million of sodium bicarbonate, with calcium omitted entirely (table I, column "30–35"). The modified solution had a P_H of 7.5. Table II shows the rapid reduction in the amount of soluble iron remaining in the solution, even when the initial concentrations were quite high. At the end of one day the amounts of dissolved iron in the flasks of Hoagland's solution depended upon the amounts originally supplied, the presence of sand having no effect. At the end of eight days, however, the flasks containing sand contained appreciably less soluble iron than those without sand. The rate at which iron disappeared from solution in the flasks containing sodium bicarbonate seems to be independent of the presence of sand.

The results presented in table II show how rapidly soluble iron salts disappear, even from a solution having an initial P_H of 5.2. At the end of one day approximately one-tenth of the iron added was still in soluble form. The amount of soluble iron is more quickly and completely reduced in solutions of increased alkalinity. These results emphasize the necessity for maintaining the supply of soluble iron by the renewal of the solutions or by the addition of iron salts at frequent intervals. A very small amount is doubtless adequate if the supply is maintained.

This whole problem has received elucidation from the work of PATTEN and MAINS (12), who have determined the H-ion concentration at which iron is precipitated from hydrochloric acid solutions by various alkalies. They found that if hydroxide is added to a dilute solution of ferric chloride, ferric hydroxide is formed if the mixture has a P_H value of 3.5. A faint cloudiness was apparent at P_H 3.5, which increased to a fine precipitate at P_H 5.5, becoming very heavy at P_H 6.0. Since it appears that iron salts become almost entirely insoluble, it becomes of interest to see what happens when the P_H of the solution is lowered by the introduction of CO_2. It is known that growing roots evolve measurable quantities of CO_2, and it has been suggested that the CO_2 evolved by plant roots is an important factor in bringing into solution otherwise insoluble iron compounds in these soils.

The following experiment was designed to throw some light upon the efficiency of CO_2 as a solvent for iron in solutions. Hoagland's solution was supplied with approximately 75 parts per million of Fe in the form of ferric tartrate and left over night. On the following morning a filtered portion of the solution contained

8 parts per million of soluble Fe. A stream of CO_2 was then passed through the unfiltered solution for several hours. At the end of that time the P_H and Fe content of a filtered portion of the solution were found to be the same as before the CO_2 was introduced. It appears that CO_2 is not able to change appreciably the reaction of a solution which had an original P_H of approximately 5.0, hence we should not expect it to increase the solubility of iron by virtue of its acid properties. The results were somewhat different when solutions of a higher P_H were employed. Culture solutions containing sodium bicarbonate were studied in a similar manner. Solution 30–35 (table I) which contained no calcium, and solution 36–41 which contained calcium, had P_H values of 7.5. Each solution received approximately 75 parts per million of Fe in the form of ferric tartrate and a small amount of solid ferric phosphate. After two hours there was no soluble Fe remaining in either solution. After the addition of another small quantity of ferric tartrate the solutions were left standing over night. On the following morning no soluble iron was found in filtered portions of either solution. A stream of CO_2 was led through each of the turbid solutions for several hours. When it was discontinued, portions of the solutions were filtered and their P_H and Fe contents at once determined. The P_H of both solutions had been changed from 7.5 to approximately 6.0, but there was no trace of soluble iron demonstrable by the colorimetric method employed.

While the introduction of CO_2 changed the reaction of the solutions, it did not increase the amount of soluble iron. In the separation of the members of the Fe-Al-Cr group in qualitative analysis, many organic substances prevent or interfere with the precipitation of iron by ammonium hydroxide. The effect of organic matter on the uptake of iron from calcareous soils is explained by COMBER (1) as being due to the solvent action of many organic compounds upon iron salts over a wide range of both acid and alkaline reactions. GILE and CARRERO (6) found that ferric citrate furnished sufficient iron for the growth of rice in either acid or neutral solutions, but that ferric tartrate furnished sufficient iron when added to alkaline solutions. As a result of their investigations it was found that these organic compounds of iron are non-toxic at the concentrations they employed. Table III summarizes the results obtained when various organic compounds were added to the nutrient solution. The culture solution "36–41" (table I) was used and had a P_H of approximately 7.5. Abundant ferric tartrate solution was added, but soon a copious precipitate settled out, and after standing over night, less than 1 part per million of iron still remained in solution. The solution was shaken and portions of the turbid solution were added to several flasks. Various organic compounds were added to the flasks, one flask being retained as a control. The flasks were then left standing a few hours. The results, while only qualitative, give a good idea of the relative amounts of iron in the filtrates after a short time interval. The object of the tests was to show the effects of various organic compounds on the solubility of iron, irrespective of their effects upon plant growth. Certain of the compounds could not be used in nutrient solutions because of their toxicity.

By supplementing the potassium sulpho-cyanide method with potassium ferrocyanide tests, it was found that glucose, sucrose, glycerine, starch, and sodium acetate each caused slightly increased solubility of iron. The filtrate from the nutrient solution which contained sodium potassium tartrate gave a deep yellow when the potassium thiocyanate method was employed in the normal manner. When the order of adding the reagents to the glass comparison tube was reversed, that is, by gradually adding the test solution to the thiocyanate and sulphuric acid, a distinct deep red was developed which almost immediately broke down to a deep yellow. When this method was employed with the filtrate from the nutrient solution treated with potassium oxalate, no red color was observed. Possibly here the decomposition of the red solution may have proceeded too rapidly to be detected. Upon adding the sodium salicylate to the nutrient solution, a clear, deep wine-red color was obtained. The conspicuous color of the iron compound thus formed has been suggested recently by COMBER (2) as a test for "acid soils."

Table III makes it evident that ammonium salts of organic acids, while they increase the solubility of iron compounds, greatly decrease the P_H of the solution. It seemed possible that the increased solubility of the iron in such cases was due largely to the decrease in P_H. Accordingly, sodium or potassium salts of organic acids were used, with the result that citrates and tartrates effected

TABLE III

EFFECT OF ORGANIC SUBSTANCES ON SOLUBILITY OF IRON IN NUTRIENT SOLUTIONS (SOLUTION 36–41)

Compound added	Appearance of contents	Color of filtered solution	Approximate P_H after adding compound and filtering nutrient solution	KSCN test for iron in filtrate
Control	Turbid (sediment)	Colorless	7.6	None
Sodium acetate	Turbid (sediment)	Colorless	7.6	Trace
Ammonium citrate	Clear	Yellow	<4.5	Abundant
Sodium salicylate	Clear	Wine red	7.6	Abundant
Ammonium tartrate	Clear (traces of sediment; no turbidity)	Yellow	<4.5	Abundant
Glycerine	Turbid	Faintly yellow	7.6	Trace
Gelatin	Turbid	Faintly yellow	7.6	Trace
Soluble starch	Turbid	Yellowish brown	7.0–7.4	Deep yellow-brown, clear; Fe present by ferro-cyanide test
Sucrose	Turbid	Faintly yellow	7.6	Trace
Dextrose	Turbid	Yellowish	7.6	Trace
Glue	Turbid	Faintly yellow	7.6	Trace
Sodium citrate	Fine turbidity	White finely divided turbidity when filtered through filter paper; clear when filtered through Chamberland filter	7.6	Abundant
Potassium oxalate	Turbid, some sediment	Colorless	6–6.5	Yellow; reversing order of reagents still gave yellow color
Sodium potassium tartrate	Clear	Colorless	7.6	Deep yellow; when reagents were reversed deep red obtained for moment, at once breaking down to yellow

marked solubility of iron without altering appreciably the P_H of the solution. Sodium acetate failed to cause an appreciable increase of dissolved iron with the tests applied, although the organic matter was destroyed before making the test. The organic compounds such as starch, sugars, etc., although not increasing very markedly the solubility of iron, nevertheless dissolved appreciable amounts of it. This is of considerable importance because a very low maintained concentration of dissolved iron is adequate for plant nutrition.

Summary

1. The iron of ferric tartrate soon becomes converted into insoluble compounds when added to nutrient solutions, and the change is more rapid in solutions of higher P_H.

2. The introduction of carbon dioxide lowers the P_H of slightly acid, neutral, or alkaline solutions, but does not increase the solubility of iron compounds which they contain.

3. The addition of certain organic compounds to an alkaline nutrient solution increases the amount of soluble iron in the solution. This fact may be of significance in maintaining an adequate supply of soluble iron in solutions for the growth of plants.

CITRUS EXPERIMENT STATION
RIVERSIDE, CAL.

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LIFE HISTORY OF ENCEPHALARTOS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 316

PAUL J. SEDGWICK

(WITH PLATES XXI, XXII AND FOUR FIGURES)

With the exception of *Bowenia*, less is known and has been published concerning the life history of *Encephalartos* than any other member of the Cycadales. In 1910 SAXTON (13) published the only morphological paper dealing solely with this genus, an account of the later embryogeny in *Encephalartos Friderici Guilielmi* and *E. villosus*. CHAMBERLAIN has made frequent incidental reference to *Encephalartos* in his various papers on other cycads, and PEARSON (12) has given valuable field descriptions in his *Notes on South African cycads*, in which he discusses three species of the genus. Before his death, PEARSON had in preparation another paper under the same title which was never completed. His notes for this paper were very kindly furnished me by Miss STEVENS of the University of Cape Town, and some further reference will be made to them. PEARSON had also contemplated an investigation of the life history, but this was not completed. CHAMBERLAIN (6) also has studied *Encephalartos* in the field. Miss SMITH (14) has estimated the number of sporangia borne on a microsporophyll of *E. villosus* as 500; and in *E. Caffer* as 700. She also gave a description of the mature microsporophylls of *E. villosus*, *E. Caffer*, and *E. Altensteinii*.

The purpose of this investigation was to establish the life history of *Encephalartos* as completely as its inaccessibility and the difficulty of securing material would permit. It was especially desired to obtain the early proembryo stages, and thus complete, with SAXTON's account of the later embryogeny, the embryogeny of *Encephalartos;* and to attempt to use this account in a phylogenetic comparison with other cycads. It was also hoped that the male gametophyte might be studied in close enough detail to permit the making of a phylogenetic comparison, but this latter hope was not fulfilled, owing to a scarcity of available stages.

Materials and methods

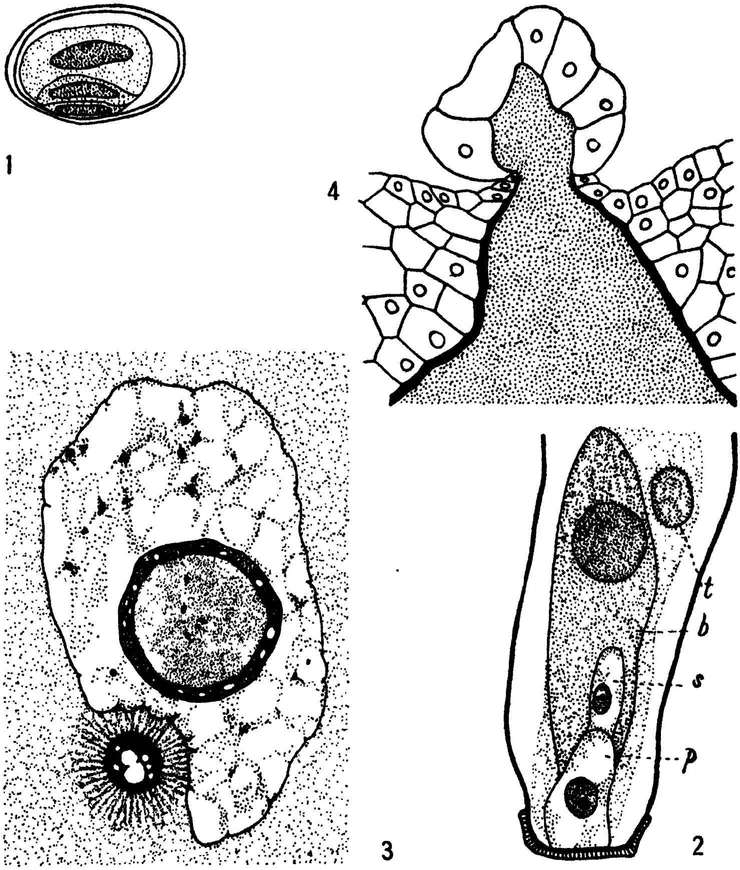
The material for this investigation was procured from a number of sources. Much of it was collected by Chamberlain during his visit to South Africa in 1912. He collected material of *Encephalartos Altensteinii*, *E. Friderici Guilielmi*, and *E. villosus.* Professor Worsdell supplied a young staminate cone of *E. brachyphyllus.* Miss Stevens sent a box of Pearson's slides of *E. Altensteinii.* Miss Van Rooyen furnished most of the material of *E. villosus* from Kentani, Transkei, South Africa. Additional material has come from the botanical garden of the University of Chicago, from Fairmount Park in Philadelphia, and from the botanical garden at Bonn, Germany. All of the material came into my possession through the kindness of Professor Chamberlain, to whom I am greatly indebted for many suggestions during the progress of the work.

Sections were made 3–12 μ in thickness with a Spencer rotary microtome, and stained in iron-alum haematoxylin with a light background of gold orange. It was found that the archegonium and proembryo could best be handled in sections 8–10 μ in thickness, while pollen grains were best sectioned at 3 μ.

Male gametophyte

At the time of the shedding of the pollen, the male gametophyte is in the usual three-celled condition. Later stages seem to be rare (fig. 1). In Pearson's slides of *E. Altensteinii* several later stages were found, but none of them was sufficiently advanced to show sperms or sperm mother cells. In this material the earliest stage shows the gametophyte of four cells, the vegetative, stalk, body, and tube cells. The haustorial portion of the pollen tube has commenced invasion of the nucellus. The prothallial, stalk, and body cells are in a line just as in *Dioon*, *Zamia*, and *Ceratozamia.* The prothallial cell bulges into the stalk cell very slightly, but the prothallial and stalk cells together press very deeply into the body cell (fig. 2).

In later stages the pollen tube is found penetrating the nucellus, but in no case was it seen to branch. The blepharoplasts have now appeared in the body cell, and from the few cases observed it is evident that the blepharoplasts appear first in the fore and aft position, and later swing through 90°, coming into a position transverse to the long axis of the tube. A detail of the nucleus of the body cell and one of the blepharoplasts is shown in fig. 3. The second ble-



Figs. 1–4.—Fig. 1, *E. villosus:* three-celled gametophyte in pollen grain; ×996; fig. 2, *E. Altensteinii:* gametophyte of four cells, prothallial (*p*), stalk (*s*), body (*b*), and tube (*t*); ×270; fig. 3, *E. Altensteinii:* detail of body cell nucleus and blepharoplast; ×996; fig. 4, *E. villosus:* archegonium with more than two neck cells (very common condition in this species), cells evidently arising from continued division of two original neck cells; ×225.

pharoplast is not present in this particular section. The blepharoplasts with their radiations are imbedded or pressed into pockets in the nuclear membrane, the appearance almost suggesting that the rays had beaten in the membrane. Later stages were not found, but the presence and position of the blepharoplasts indicate that division of the body cell is close at hand, and that two sperms will be formed.

Female gametophyte

The megaspore, the first cell of the female gametophyte, has been observed in only three of the nine genera of cycads. In *Stangeria paradoxa*, LANG (11), and in *Ceratozamia longifolia*, TREUB (17), describe an axial row of three "megaspores," of which the innermost one is functional. In *Zamia floridana*, Miss SMITH (15) figures an axial row of four megaspores, the innermost one functioning. In the cycads it is exceedingly difficult to obtain such early stages, because the young cones are completely concealed by the bud scales, and it is impossible to determine from superficial examination whether the bud contains a cone, or only a new crown of leaves.

In the present material the earliest stage available shows free nuclear division of the gametophyte. The ovules from which the sections were made had been pickled whole, and the gametophytes had shrunken away from the periphery of their cavities. The approximate dimensions of the cavity are 22.5 mm.×12 mm. The layer of cytoplasm containing the imbedded nuclei is very thin in proportion to the huge vacuole, being 50μ thick. The nuclei are in one layer. While the gametophyte is still in an early free nuclear condition, organization of the pollen chamber is indicated in the nucellus above the gametophyte. The pollen chamber appears first as a region in the central portion of the nucellus, well below the nucellar beak, where the cells in a vertical line are becoming separated from their neighbors through middle lamella resorption (fig. 14).

Early stages of wall formation in the gametophyte have not been found, the next stage showing the nearly mature gametophyte with central cells nearing the time for division. In these archegonia the primary neck cell has already divided to form the two neck cells.

Occasionally the two neck cells divide, giving rise to several cells, as shown in fig. 4. It is possible in numerous instances to observe the behavior of the central cell nucleus in preparation for division. At the time when radiations in the cytoplasm are lining up and attaching themselves to the nuclear membrane, showing clearly that division is imminent, the central cell nucleus is very large in proportion to the contained chromatin and other material (fig. 7). The nucleus seems to have a topography resembling that of a nucleus in synapsis, and the contracted appearance, as in synapsis, is due largely to a rapid increase in the size of the nuclear cavity, rather than to any contraction of the chromatin. Most of the nuclear cavity is filled with a homogeneous plasm and ten or a dozen roughly spherical, light staining bodies with dark centers. The chromatin and another material (for it does not seem probable that the great quantity of darkly staining material is all chromatin) are in a close spirem. That not all of this material is chromatin is shown by a study of the figure for the division of the central cell. Until earlier stages were located this topography was puzzling. In the younger central cell nucleus there is no such disproportion between the volume of the whole nucleus and the volume of contained chromatin and other undetermined darkly staining material. The volume of the nucleus increases by a peculiar engulfing of general cytoplasm, the nuclear membrane bulging inward. Later the membrane heals over and the engulfed membrane must be resorbed, dissolved, or otherwise disappear. Furthermore, it seems that certain food bodies which are found to be abundant in the cytoplasm of the central cell are taken into the nucleus in the same manner, and these are the roughly spherical, dark centered bodies previously mentioned. The process bears a very close resemblance to the behavior of the male and female nuclei of *Peperomia* in the absorption of protoplasm, as described by BROWN (1). The occasion for this great growth of the central cell nucleus is not understood (figs. 5–7).

As already mentioned, the central cell nucleus, which so resembles a nucleus in synapsis, has already begun to show radiations in the cytoplasm, indicative of its early division. Details of the division were not observed, the next stage found showing the egg nucleus and ventral canal nucleus at telophase, with membranes forming.

Prominent spindle fibers connect the nuclei, and polar radiations are very distinct. There is no evidence of even an evanescent accumulation of wall material on the spindle fibers. Thus *Encephalartos* is similar in this respect to all the cycads so far studied. In the division observed, the ventral canal nucleus was considerably smaller than the egg nucleus, seeming to indicate that it would very shortly degenerate. Either this was not a normal division, or this assumption is incorrect, because in most cases the ventral canal nucleus was not observed to degenerate promptly, as is usually the case in cycads. Instead, the ventral canal nucleus enlarges rapidly and seems to go through a maturation phase similar to that of the egg (figs. 8, 9). Here again there is some evidence that the ventral canal nucleus grows by the amoeba-like intake of food and protoplasm described for the central cell nucleus, instead of simply by osmotic absorption, as must naturally be assumed in the absence of such a condition. In the case of the ventral canal nucleus growth, however, available stages are not so good as the stages showing the growth of the central cell nucleus.

Embryogeny

Fertilization was not observed, and apparently fertilization by a sperm is not necessary in *Encephalartos* for the successful development of embryos. In greenhouse material and material collected in the field it is found that the ventral canal nucleus, instead of degenerating, has enlarged and is approaching the egg. The first case of fertilization described for gymnosperms by STRASBURGER (16) for *Picea*, and the second case, described for *Pinus* by COULTER (7), were of this character (figs. 8, 9). CHAMBERLAIN (3) has reported a similar condition as occurring occasionally in *Ceratozamia* and *Encephalartos*. He concludes that a union of the two nuclei is very probable. The present observations support this conclusion. While the two nuclei were not actually found in union, they were very close together. In his paper on *Ceratozamia mexicana*, CHAMBERLAIN (3) believes that this situation throws an explanatory light on VAN TIEGHEM'S reported hybrids, a cross between *C. longifolia* and *C. mexicana* in which three year old pollen of *C. mexicana* was used. It is not believed that cycad pollen will live three years. CHAMBERLAIN thinks that either the ventral canal nucleus united with the egg nucleus, or that the embryo was produced without a union.

An additional reason for concluding that fertilization by a male nucleus may not be necessary is based on CHAMBERLAIN'S field observations. In collecting *Encephalartos Friderici Guilielmi*, during a whole day's search, with the aid of four assistants, he procured only one staminate cone in the neighborhood of plants bearing ovulate cones. A further reason for concluding that sperm fertilization is frequently lacking is found in careful study of all the preparations. In not a single free nuclear proembryo has a sperm sheath or ciliated band been found. In the studies previously made by LANG (11) and CHAMBERLAIN (2) it is reported that the sperm sheath and the ciliated band persist in the cytoplasm of the cycad proembryo up to a very late stage of free nuclear division, and often even after cell walls have begun to appear. The cytoplasmic sheath of the sperm, and especially its ciliated spiral band, are so conspicuous that failure to find them in complete serial sections is practically a proof that they are not present. In view of these three considerations, therefore, it is safe to conclude that *E. villosus* and *E. Friderici Guilielmi* can produce normal embryos in the absence of pollination and fertilization by a sperm. This does not mean that sperm fertilization never takes place, but simply that the preparations examined do not show any indication of sperm fertilization, and they do show proembryos and embryos which seem to be normal. PEARSON (12 and unpublished notes) thought it probable that *E. villosus* is insect pollinated, since the coming specimens were frequently so situated that it is unreasonable to suppose that wind could carry the pollen and deposit it in sufficient quantity to account for the numerous embryos. Furthermore, he found certain species of the weevil, *Phaeophagus*, constantly associated with the cones, and the bodies of the weevils are frequently smeared with pollen. There is also similar evidence for belief that *E. Friderici Guilielmi* and *E. Altensteinii* may be insect pollinated. Observations showing that fertilization is not necessary would permit that coming specimens be isolated, and thus make it unnecessary to search for insect pollination. It is very possible, however, that when normal pollination and fertilization occur, the pollen may be carried as suggested by PEARSON. CHAMBERLAIN, however, believes that there is no insect pollination in any of the cycads.

The earliest free nuclear divisions of the proembryo were not observed. The latest free nuclear stage found seemed to have a fairly close approximation to the 1024 nuclei which would result from the tenth simultaneous division (fig. 11). Neither in this stage nor in any of the preceding stages was an evanescent segmentation, such as CHAMBERLAIN (2, 5) describes for *Stangeria* and *Dioon edule*, observed. Since the division figures were not found, it is impossible to say whether such an evanescent segmentation occurred. It is possible to conclude that since there is no segmentation with the nuclei of the tenth division in the resting condition, either another division will take place or cell walls are not formed in the usual manner.

When next observed, the proembryo is made up of cellular tissue, with definite permanent cellulose walls. The suspensor has started to elongate and differentiation of the embryo has begun. There is some indication of the breaking down of the cellular tissues at the center of the proembryo (figs. 12, 13). The next stages show the elongating suspensor, twisted in the embryo cavity and now backing up into the empty archegonium. The development of the embryo proper has been described by SAXTON (13), and will not be taken up here, except to remark that the cotyledons and stem tip can be distinguished in the present material considerably earlier than in any figured by SAXTON. His earliest stage shows the proembryonal tissue completely broken down, while this material shows a differentiation of cotyledons and stem tip before the proembryonal tissue has completely disorganized.

It is interesting and suggestive to compare the embryogeny of *Encephalartos* with the embryogeny of other cycads. *Cycas*, *Zamia*, *Ceratozamia*, *Dioon*, *Stangeria*, and *Macrozamia* have been investigated, *Zamia* and *Stangeria* receiving the more thorough attention. In *Macrozamia Moorei* and *E. Friderici Guilielmi* segmentation is complete and permanent, cellulose walls being formed throughout the proembryo. Later a cavity forms by the breaking down of the cellular tissue. COULTER and CHAMBERLAIN (9) believe, from examination of the figures on *Cycas* by IKENO (10) and TREUB, and from slides made from material furnished by IKENO, that segmentation is also complete in *Cycas*. If this is correct, it follows that the figures given by IKENO and TREUB are of rather an advanced stage in the breaking down of the cellular tissue. In *Dioon edule* and *Stangeria paradoxa*, evanescent walls appear throughout the entire proembryo, but permanent walls are formed only at the base. In *Zamia floridana*, COULTER and CHAMBERLAIN (8) found that segmentation takes place only at the base. *Bowenia* and *Microcycas* have not been investigated. The question naturally arises as to whether the development of the proembryo shows any evolutionary significance. It is generally agreed that *Cycas* is the most primitive living cycad, this conclusion being based largely on the leafy character of the ovulate sporophyll, and the absence of a definitely organized ovulate strobilus. *Macrozamia Moorei* is also accorded a primitive position, on the basis of an entirely different character, namely, the possession of numerous lateral cones, and, associated with this condition, the absence of cone domes. Furthermore, the leaf trace is direct. *Encephalartos*, like *Macrozamia*, possesses numerous lateral cones, but by no means so great a number. Thus there is evidence for primitiveness in *Encephalartos* just as in *Macrozamia*. On the other hand, it has long been agreed that *Zamia* is the most advanced of the cycads. Its compact cones, the practically entire loss of leaflike character of sporophylls, the reduced number of microsporangia, the girdle of leaf traces, and the possession of cone domes, all testify to the advanced position of *Zamia*. So the evolutionary series, based on external characters and gross morphology, runs from *Cycas* through *Macrozamia*, *Encephalartos*, *Stangeria*, and *Dioon* to *Zamia*, and a study of the embryogeny provides a distinct confirmation. In the primitive genera the proembryo becomes completely cellular (*Encephalartos*, *Macrozamia*, and *Cycas*); the next stage in evolution is where segmentation is evanescent except at the base from which suspensor and embryo are organized (*Dioon*, *Stangeria*); while in the final stage, as shown by *Zamia*, segmentation never takes place except at the base. It will be interesting to learn the embryogeny of *Microcycas* and *Bowenia*. If this theory of the evolution of the embryogeny is correct, we should expect *Bowenia* to show complete segmentation with walls, perhaps permanent, but more probably evanescent.

Microcycas presents such a mixture of advanced and primitive characters that it would be entirely unsafe to guess at its embryogeny. Cycad embryogeny from an evolutionary standpoint has been discussed by CHAMBERLAIN (6).

Summary

1. An extensive free nuclear period follows the germination of the megaspore.

2. The pollen chamber begins to form within the nucellus while the gametophyte is still in an early free nuclear stage. It appears first in the central portion of the nucellus below the nucellar beak.

3. The male gametophyte has prothallial, stalk, body, and tube cells. The body cell was observed with two prominent blepharoplasts. The pollen tube is unbranched.

4. The central cell nucleus enlarges greatly before its division to form the ventral canal and egg nuclei. Increase in the volume of the nucleus takes place by the bodily inclusion of masses of cytoplasm.

5. The ventral canal nucleus frequently does not promptly degenerate, but enlarges, goes through a period of maturation very similar to that of the egg, and passes toward the egg, suggesting a probability of fusion of egg and ventral canal nuclei.

6. No proembryos were found containing sperm remnants, indicating that fertilization by a sperm is not necessary for the production of the proembryo.

7. The 1024-nucleate stage of the proembryo shows neither walls nor evanescent segmentation. Evanescent segmentation may have occurred following this tenth division, but its disappearance or its failure to appear, whichever is true, indicates that another division is to take place.

8. The proembryo becomes completely cellular. Later a vacuole forms by the breaking down of the central portion of the proembryonal tissue; eventually this tissue is completely resorbed.

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EXPLANATION OF PLATES XXI, XXII

Fig. 5.—*Encephalartos villosus:* central cell nucleus taking in cytoplasm; pore not yet closed over; ×251.

Fig. 6.—*E. villosus:* central cell nucleus taking in cytoplasm; ×251.

Fig. 7.—*E. villosus:* central cell nucleus shortly before division; ×324.

Fig. 8.—*E. villosus:* ventral canal nucleus enlarging and moving toward egg nucleus; ×32.

Fig. 9.—*E. villosus:* ventral canal nucleus moving toward egg nucleus; ×32.

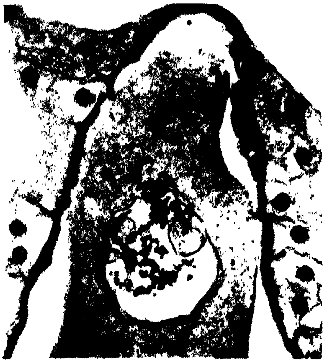
Fig. 10.—*E. villosus:* an unusual archegonium with very long neck region; ×80.

Fig. 11.—*E. Friderici Guilielmi:* free nuclear proembryo (approximately 1024 nuclei at this stage); ×26.

Fig. 12.—*E. Friderici Guilielmi:* cellular proembryo; vacuole forming at center through breaking down of cells; ×69.

Fig. 13.—*E. Friderici Guilielmi:* suspensor and embryonal region; from same section as fig. 12; ×69.

Fig. 14.—*E. brachyphyllus:* origin of pollen chamber through disorganization of cells in central portion of nucellus; at this time gametophyte is in early free nuclear condition; ×50.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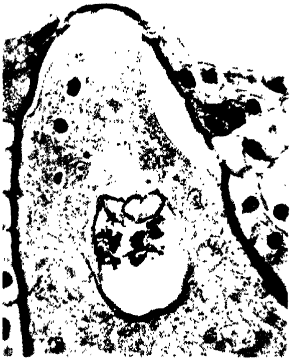
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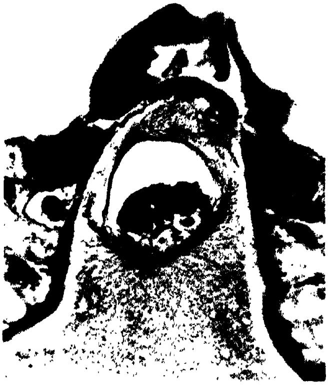
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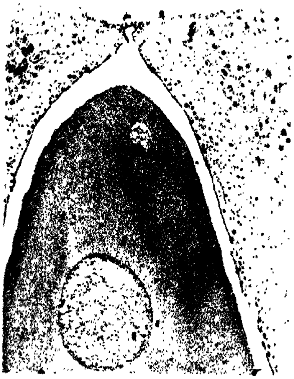
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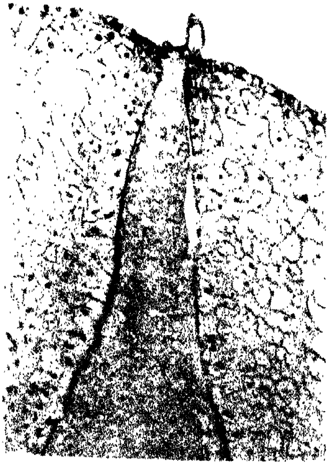
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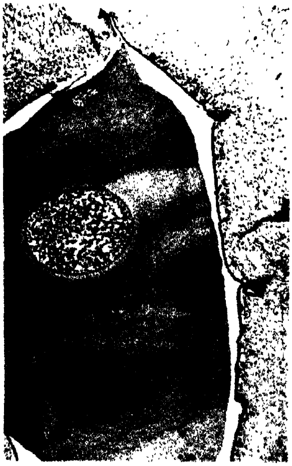
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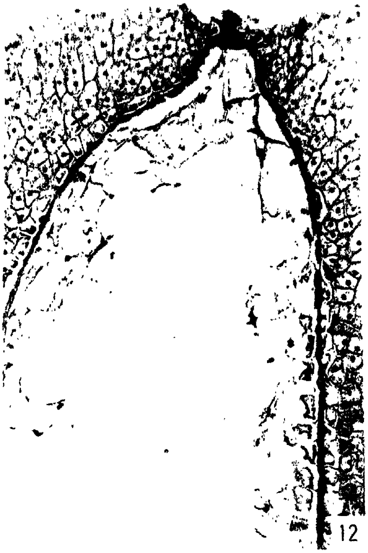
SEDGWICK on ENCEPHALARTOS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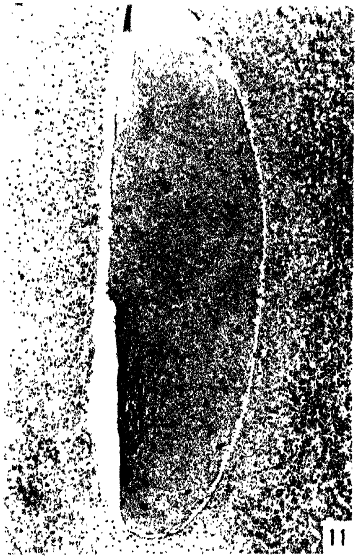
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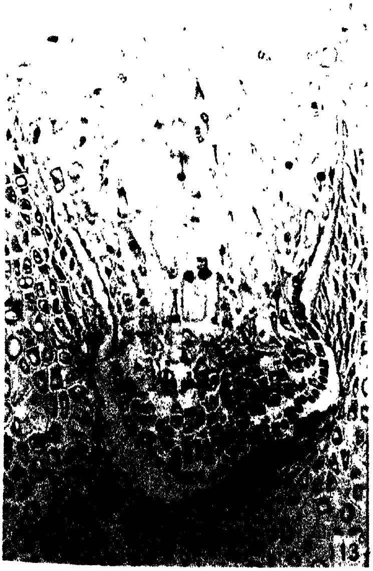
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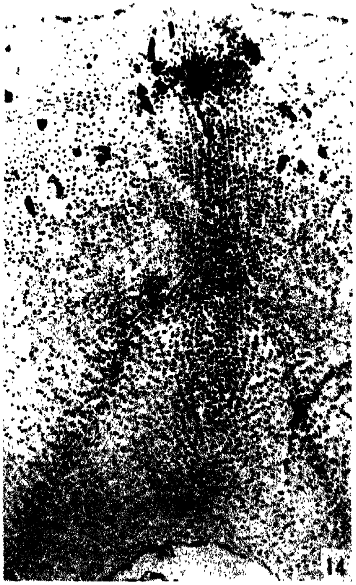
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SEDGWICK on ENCEPHALARTOS

ASSIMILATION-RESPIRATION BALANCE AS RELATED TO LENGTH OF DAY REACTIONS OF SOY BEANS

FRANK M. EATON

(WITH FOUR FIGURES)

Considerable interest has recently been directed toward the seasonal behavior of plants by the work of GARNER and ALLARD.[1] These investigators have laid particular stress upon the relative length of day and night as the factor which directs plant growth to the vegetative and reproductive stages. Later HARVEY[2] has shown that a wide range of plants may be brought to sexual maturity under conditions of uniform temperature and continuous artificial light. HOPKINS,[3] in his statement of the bioclimatic law, would include besides length of day (latitude) all of those climatic factors which are influenced by longitude and altitude. It is doubtless true in nature that the seasonal changes in the development of vegetative and reproductive characters correspond most closely to changes in the relative length of day and night. One of the chief functions of a plant peculiar to the hours of daylight is the assimilation of carbon dioxide. In the absence of light this process is found measurably reversed through respiration.

The somewhat preliminary experiments to be described here were planned with a view toward finding whether the daily balance between these two plant processes, assimilation and respiration, might not stand as a controlling influence which could direct plant growth either to a vegetative or reproductive form. If this were the case, then it would be expected that lower daily temperatures, increased nightly temperatures, or a reduction in the number of hours of the day during which carbon dioxide was available for assimilation would bring about changes in the behavior of plants similar to those which follow a decrease in the length of day.

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Nightly temperatures can be controlled experimentally with comparative ease and with little disturbance of the other external factors upon which plant growth is dependent. This method was used first and most extensively. Later, as a check upon the results so obtained, a few plants of Peking soy beans were deprived of carbon dioxide for a number of the hours of the day. Soy beans were used as a principal test plant because GARNER and ALLARD had already so thoroughly demonstrated their length of day responses.

Effect of different nightly temperatures upon time of flowering of Peking soy beans

Three sets of twelve plants of Peking soy beans were given a thirteen-hour day out of doors. During the nights one set of these was placed in a chamber with a temperature of about 50° F., the second set in a control chamber at about 65°, and the third was given a nightly temperature of 90°.

TABLE I

PEKING SOY BEANS PLANTED JUNE 20 (SPROUTED SEED): UP JUNE 24; TREATMENT STARTED JUNE 20, 1922

	Cold	Check	Hot
Days from emergence to flowering.....	45.0	25.0	21.0
Unfolded leaves, 21st day (July 15)....	6.0	6.2	7.3
Height (cm.).......................	7.7	8.6	8.6
Leaves, 45th day (August 8)..........	11.1	9.0	9.0
Height (cm.) 45th day (August 8).....	32.0	25.0	28.0
Height (cm.) at maturity.............	60 0	25.0	28.0
Total dry weight, plant and beans.....	292.4	81.8	122.2

The differences in the time of flowering of these sets of plants were almost as great as the difference effected by GARNER and ALLARD by exposing plants to varied lengths of day. The set given the cold nights flowered on the forty-fifth day after germination, those in the check chamber on the twenty-fifth day, and those in the hot night chamber on the twenty-first day (fig. 1). Untreated plants grown in the field under a full day flowered three days earlier than did the plants given the cold nights, the latter receiving only the thirteen-hour day. The behavior of these plants is shown more completely in table I.

The soy beans used in this experiment were grown in wooden boxes four feet long and one foot square. They were placed on trucks and pushed into the respective temperature control chambers at 7 P.M. and removed each morning at 6 A.M. The temperature of the cold night chamber was reduced by circulating the air of the chamber through an ice chest filled with cracked ice. The temperature of the check chamber was uncontrolled. Electric heating elements with thermostatic control and a circulating fan were used for heating the hot chamber.

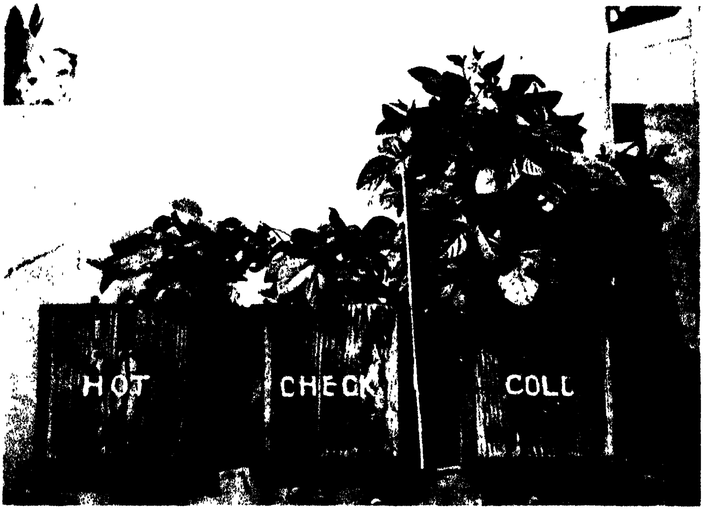

FIG. 1.—Peking soy beans, showing effect of hot and cold nights upon growth

MEASUREMENTS OF ASSIMILATION AND RESPIRATION.—As the foregoing results were quite outstanding, it seemed desirable that a quantitative estimate of the respiratory rates of the plants in the various chambers and the ratio of assimilation to respiration be secured. While time and equipment were not available for the development of elaborate technique, data of some interest were secured.

For the measurement of both assimilation and respiration, rooted plants were transplanted to trays four inches deep and one foot square. Over the top of these trays a heavy plate glass with a ground surface was placed. In the center of each plate there was a one-inch hole through which the stalk of the plant extended. The space between the plant stalk and the edge of the opening was filled with wax, forming an air-tight seal between the soil below and the air above. While measurements were being made the plants were covered with bell jars which were sealed to the ground glass plate. In measuring respiration the plants were placed in the respective temperature chambers at 7 P.M. and removed the following morning at 6 A.M. From each of the bell jars there were two tubular openings connected with the outside of the chambers by rubber tubing. Before removing the plants at 6 A.M., the air in the bell jars, which then contained the additional CO_2 of respiration, was drawn off for a minimum period of one hour and passed through barium hydroxide bead towers, the barium hydroxide later being titrated with tenth normal hydrochloric acid. By estimate five volumes of air were removed from the bell jars for each determination. The air as removed was replaced with carbon dioxide free air. Assuming that each volume of air removed halved the CO_2 content of the bell jar, then one thirty-second of the CO_2 would still have remained, plus a portion of the carbon dioxide respired by the plant during the last hour. This error is not accounted for in the results, but the original CO_2 content of the normal air in the bell jar when the plant was covered has been subtracted.

The assimilation measurements were made out of doors with plants which had been used for respiration measurements. A stream of air, as nearly constant in its rate of flow as possible, was provided by means of a valve opening into a large vacuum tank connected to a motor-driven exhaust pump of sufficient size to maintain the partial pressure of 12 cm. mercury in the tank. For a fifty-minute period the air entering the valve was drawn from the bell jar containing the plant and passed through a barium hydroxide bead tower. For the succeeding ten-minute period normal air was drawn through a second bead tower. This alternating process was continued for a thirteen-hour day. The barium hydroxide of the respective bead towers was titrated and renewed at the end of each period. The CO_2 content of the normal air passing through the bead tower for the ten-minute period was determined and multiplied by five. This value was taken as the equivalent of the CO_2 supplied to the plant during its fifty-minute period. The amount of CO_2 leaving the bell jar, subtracted from the amount which entered, gave the difference representing the CO_2 assimilated by the plant for one hour.

TABLE II

ASSIMILATION AND RESPIRATION MEASUREMENTS, PEKING SOY BEANS

Plant	Age in days	Mean temperature	Dry weight (gm.)	Leaf area (sq. cm.)	*Respiration:* gm. of CO_2 per 100 gm. (11 hours)	*Assimilation:* gm. of CO_2 per sq. m. of leaf area (13 hours)
A*	31	48	....	....	0.407	....
	32	47.5	....	....	0.462	....
	33	55.5	12.5	....	0.541	....
B*	31	91.4	....	....	0.890	....
	32	94.0	....	....	0.850	....
	34	90	10.5	....	1.112	....
C	18	47.5	....	....	1.28	....
	19	47.5	0.78	....	1.28	....
D	18	90.5	0.73	....	2.48	....
E (2 plants)	9	63	0.315	....	2.17	....
F†	12	53.3	0.165	....	2.19	....
	13	82.8	....	18.42	....	29.32
G†	12	85.1	0.175	....	3.17	....
	13	84.3	....	28.32	....	27.89

* Plants A and B were cut and dried six days after the last respiration measurements were made.

† Assimilation measurements for plants F and G were made on the same day. Their dry weights were nearly the same, they had the same number of leaves, but on measurement it was found that the leaf area of the one was much greater than the other.

To control the temperature inside the bell jars during the middle hours of the day it was found necessary to erect a cheesecloth shade above the plants. The temperature as read from a thermometer with a protected bulb was not allowed to go above 90°.

The results given in table II indicate that respiration proceeded almost twice as rapidly in the hot night chamber as it did in the cold night chamber, and that a ratio of about fifteen to one existed between assimilation and respiration. Assimilation measurements were made on some of the larger plants, but due to their size, the air passing through the bell jar was so depleted of carbon dioxide (to the extent of 50 per cent) that the results obtained were probably too low. In the cases given the CO_2 content of the air leaving the bell jar was 70–80 per cent of normal during the middle hours of the day. From the last two plants, F and G, it appears that assimilation was little affected by the temperature of the previous night, although the respiration of the hot night plant was almost double that of the plant in the cold chamber.

PHYSIOLOGICAL NIGHT.—In this experiment a second method of disturbing the assimilation respiration balance was tried. The plants were deprived of carbon dioxide for a number of hours each day. It was not possible to conduct this experiment until late in the season, by which time outside temperatures were too low for soy beans and the length of day too short for normal vegetative development. For these reasons the plants were grown in the greenhouse and electric lights used to supplement the normal length of day.

Four groups of Peking soy beans were planted in a tray October 4. They germinated October 13, and after the second day were given thirteen and one-half hours of light by supplementing sunlight in the late afternoon and evening with a 300 watt incandescent lamp, with a shade, suspended two and a half feet above the plants at germination. On and after the ninth day from germination, one group of these plants was given a seven-hour day by covering them with a light-proof earthenware jar between the hours of 3 P.M. and 8 A.M. A second group was inclosed in a glass bell jar above the plate already described, and supplied only with air from which the carbon dioxide had been removed. This treatment was continued during the same hours for which the first group of plants was covered with the light-proof jar. The bell jar was removed during the day. A third group was untreated, and a fourth group covered with a bell jar held above the soil, leaving a space of several inches at the bottom to admit air freely, but at the same time to simulate to some extent the temperature and light conditions which the plants under the other bell jar were receiving. The plants given the short days produced flowers on November 28. None of the plants in the other groups had flowered by December 24, at which time the treatment was discontinued. The growths of the plants given the long dark night and of those given the "physiological night" were very similar, the plants attaining average heights of 10 and 13 cm. respectively. The other plants made a much greater growth than did the first two groups, the heights being 33 and 37 cm., the group partially inclosed within the second bell jar being again the taller.

If the premise of this second experiment is correct, it is possible, and perhaps probable, that the results obtained in the first experiment were purely the effects of nightly temperatures. If so, nightly temperature in its action upon plants does not produce the same response with all species, even though these species may react alike to shortened days. For this reason there are appended the following notes on the behavior of other plants, which were also given the higher and lower nightly temperatures. While the preceding has to do with nightly temperatures, there may be no special reason for believing that increased or decreased temperatures at another time than during the night might not also bring about differences in the growth and development of plants.

Effect of high and low nightly temperatures upon other plants

COTTON (Durango).—Under the cold night treatment the plants developed only a few tiny aborted leaves above the cotyledons, and then died. The cotton in the hot chamber flowered on August 7 (planted June 13). The plants bore heavily, and the growth was of a fruiting type. The check night plants made about the same growth, but it was of a more vegetative type; only a few bolls were set, these coming from flowers which opened in September, four days after the treatment had been stopped (fig. 2).

COSMOS (Lady Lenox and Klondike).—These plants flowered when given the thirteen-hour days in both the check and hot chambers. The plants in the check chamber were somewhat earlier than were those given the hot night, and the growth was more normal, and more dry matter was produced. Klondike cosmos in the cold night chamber made only a very feeble growth, and did not show signs of flowering until after the treatment was stopped. These varieties of cosmos are short day plants (fig. 3).

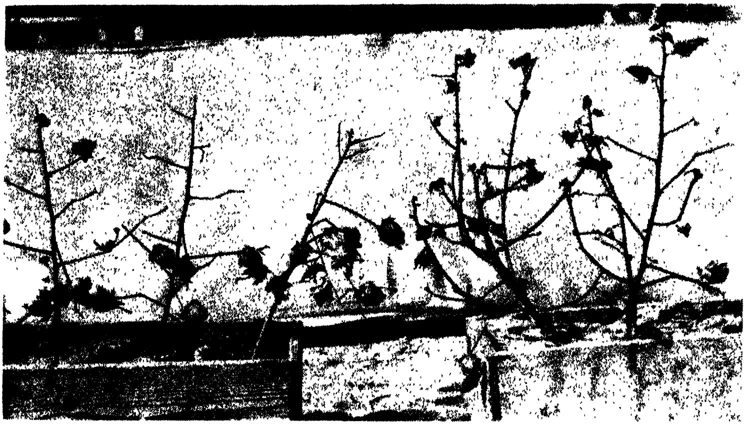

FIG. 2.—Durango cotton, showing effect of high night temperature (left with bolls) compared with check at right (leaves removed).

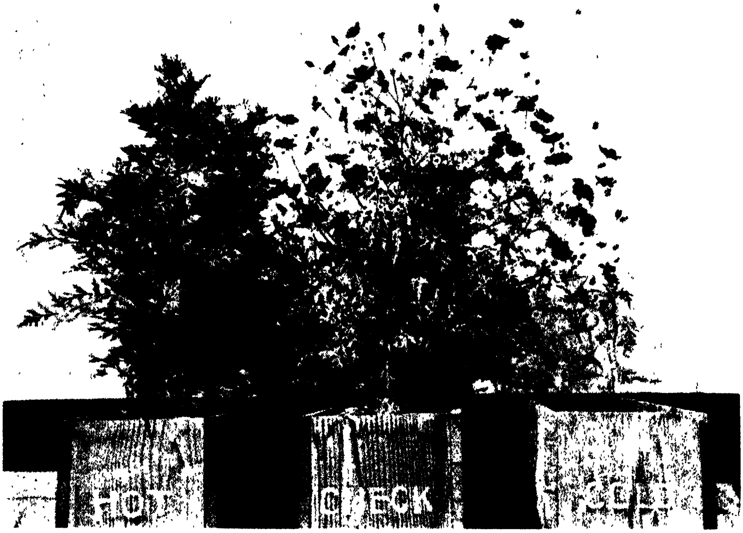

FIG. 3.—Klondike cosmos, showing relation of temperature treatment to blooming and growth.

Phlox (perennial).—Well started roots, treated after May 9, flowered in the hot chamber July 7, in the cold chamber July 28, and in the check chamber August 7. There was only one plant of each.

Aster (Crego).—Young plants were transplanted and treated after May 9. The plant in the hot chamber flowered August 10, in the check chamber August 29, and in the cold chamber September 1. Plants in the field flowered August 13.

Red clover.—These were planted in the early spring and treated after May 9. They did not flower in either the cold or check chamber. Two plants out of eight grown in the hot night chamber flowered July 22. Field-grown plants flowered July 22.

Lemon trees (Lisbon variety, on Sour Orange stock).—These young shoots from fall buds made in the cold chamber 37 cm. growth, check chamber 69 cm., hot chamber 46 cm., in the field with a normal day 76 cm., and with a 10-hour day 39 cm.

Potatoes (McCormick).—These were not started under the treatments until they were almost ready to flower. The plants in the hot chamber were less coarse, more upright, and made a greater top growth than did the plants in the cold or check chambers. These latter plants matured earlier than did the hot night plants. In yield there was very little difference, but when harvested the tops of the hot night plants had not started to die.

Zea Mays (Navajo).—Fig. 4 shows the hourly elongation of maize under different nightly temperatures. These elongation curves have no direct bearing upon the assimilation-respiration balance theme, but they are of some interest from the standpoint of the effect of nightly temperatures. The plants from which these elongation measurements were made were grown in the greenhouse in the fall of the year. The "hot" and "cold" nightly temperatures were about the same as for the summer experiments, but the temperature of the uncontrolled check was considerably lower.

Summary

1. The time of flowering of Peking soy beans given high, low, and uncontrolled nightly temperatures was affected to an extent comparable with the differences brought about by varying the length of day.

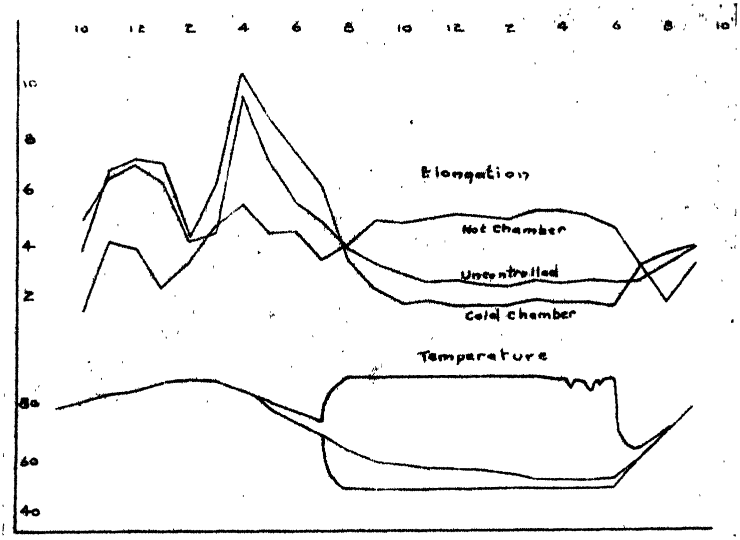

FIG. 4.—Elongation of maize given hot, cold, and uncontrolled temperatures during night; elongation in mm. per hour shows close relationship to nightly temperature.

TABLE III

MAIZE

	Cold	Check	Hot	Field
	Navajo, planted April 25, up May 3; treatment May 9–July 20			
Days "up" to pollen	64.4±0.45	54.6±0.31	55.4±0.41	....
Days pollen to silk	1.6±0.56	0.0±0.36	7.0±0.56	....
Total number of leaves	14.0±0.0	12.2±0.12	15.4±0.24	....
Length of longest leaf	73.0±1.09	72.8±0.99	76.2±0.72	....
Width of longest leaf	6.4±0.21	7.2±0.13	7.1±0.15	....
	Northwestern Dent, planted July 10, up July 17; treatment started July 20			
Days "up" to pollen	45.8±0.36	38.6±0.49	39.0±0.47	40.3±0.46
Total number of leaves	12.8±0.18	12.4±0.31	16.2±0.40	14.3±0.16

2. It was found by measurements that respiration proceeded almost twice as rapidly under hot night conditions as under cold night conditions.

3. Depriving soy beans of carbon dioxide for a number of the hours of the day did not affect the time of flowering.

4. Not all short day plants gave the same reactions to varied nightly temperatures.

5. The elongation of maize during the night was found to be nearly proportional to the temperature.

The writer wishes to express his thanks to Mr. G. N. COLLINS, for his helpful suggestions during the course of these experiments, and to Dr. R. B. HARVEY, for his suggestions on the preparation of the data for publication.

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EFFECT OF ETHYLENE UPON RESPIRATION OF LEMONS[1]

F. E. DENNY

(WITH TWO FIGURES)

When lemons are received at the packing house, some of them, particularly in certain seasons, are green in color. Such fruit is regarded as commercially mature, and, in fact, represents a high market grade when the yellow color has been brought out by subsequent treatment. If these green lemons are sorted out, placed in boxes, and stored under suitable conditions, such as are obtained in basement storerooms, a yellow color develops in from one to two months. Practical experience has shown that the desired color can be obtained in from one to two weeks by placing the fruit in rooms heated with kerosene stoves. The experiments of SIEVERS and TRUE (6) showed conclusively that the coloration of the fruit in this forced curing method was brought about, not primarily by the conditions of temperature and humidity existing in the room, but by the gaseous combustion products generated by the kerosene stoves. As a result of this discovery, the common method employed at present consists in placing the kerosene burners in a separate building, called the generator room, and conveying the gaseous products to the various sweat rooms by conduits and electric fans.

Experiments were started by the Laboratory of Fruit and Vegetable Chemistry, Bureau of Chemistry, United States Department of Agriculture, to determine, if possible, the gaseous constituent of the sweat room atmosphere that was responsible for coloration of the fruit. Details regarding these experiments will not be described in this paper, but are to be published in a succeeding article. It is sufficient to state that ethylene was very effective in bringing about the desired result, concentrations as low as one part (by volume) of ethylene in one million parts of air being sufficient to cause green lemons to turn yellow in about six to ten days.

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[1] Published by permission of the Secretary of Agriculture.

The experiments also showed that high concentrations of ethylene (80 per cent), high temperatures (92° F.), low temperatures (45° F.), and lack of oxygen either entirely prevented or greatly delayed coloring. Conditions suitable for the life processes of the fruit were favorable for coloring. Furthermore, coloring with either ethylene or gas from the kerosene stoves caused the loss of the "buttons" (calyx, receptacle, and a portion of the peduncle). A microscopical examination of the cells at the absciss layer showed that they had become enlarged and gelatinous. Apparently secondary growth had started in this area at least, producing a condition in lemons analogous to the extrusion of tissue on stems of various plants mentioned by Miss DOUBT (1).

These facts indicated that ethylene in some manner stimulated the growth, or the life processes of the cells of the fruit. If this were the case, the respiration of the fruit should be increased. The results of the measurements of the carbon dioxide output of ethylene treated lemons as compared with those receiving no ethylene are here recorded.

Methods

Fresh green lemons from a commercial packing house were selected for soundness and uniformity. They were sorted into lots of six or seven lemons and placed in glass desiccators (6–7 inches in diameter) provided with inlet and outlet tubes, arranged to draw a current of air through the system. Rubber stoppers were firmly tied and all rubber connections were covered with paraffin. The desiccators containing the fruit were kept in an incubator at 25° C. continuously, except for the periods during which air was being aspirated through them for the absorption of the carbon dioxide. At such times it was necessary to expose them to the laboratory temperature, which varied on different days from 18° C. to 22° C.

Once each day the fruit was thoroughly aerated, in one experiment by removing the desiccator covers for one-half hour and exposing the fruit to the outdoor air, and in another experiment by removing the fruit from the container, filling the container with water, emptying it, and replacing the fruit. Starting from the time of complete aeration, the carbon dioxide was allowed to accumulate, in the first experiment for 2.5 hours, and in the other for 1.5 hours.

The carbon dioxide was absorbed by a solution of barium hydroxide placed in Pettenkofer tubes, as recommended by GRAFE (2). Bubbles were aspirated through at the rate of about 2 liters per hour, in the first experiment for 2.5 hours, and in the other for 1.5 hours. The reduced pressure was regulated to about 1.3 cm. of mercury below atmospheric by a Palladin pressure regulator (GRAFE 2). Carbon dioxide was removed from the incoming air by soda lime tubes, and the outgoing air was tested for complete absorption by a bubbler of barium hydroxide.

At the end of the absorption period, the barium solution was washed into volumetric flasks with carbon dioxide-free water, made up to volume, and after the carbonate had settled to the bottom an aliquot of the supernatant solution was removed. The residual barium hydroxide was titrated with sulphuric acid standardized to a strength such that 1 cc. was equivalent to 1 mg. of carbon dioxide (OLSEN 5), using phenolphthalein as indicator. Each day a blank experiment with a desiccator containing no fruit, but to which was added one part ethylene to one thousand parts of air, was run. Titration values obtained with fruit were deducted from the titration values of this blank. Results were calculated to milligrams of carbon dioxide per kilogram of fruit-per-hour basis. Time was counted from the sealing of the desiccator to the end of the absorption period.

Effect of ethylene upon respiration

Lemons were divided into four lots. In two of the desiccators ethylene was added from a gas burette in quantities sufficient to make the concentration of ethylene within the desiccator approximately one part in one thousand, account being taken of the volume occupied by the fruit. The subsequent treatment of the different lots is shown in table I, the data of which are shown graphically in fig. 1.

At the end of the first day, the respiration of the ethylene treated lots was decidedly increased, although both lots did not respond equally well to the treatment. Lot 849 was given ethylene at a concentration of 1 part in 1000 at the end of the second day, and two days later the respiration had increased about 175 per cent.

After the respiration of lot 821 had been increased by the use of ethylene, a discontinuance of the ethylene applications led to a decrease. The rate of decrease after discontinuing ethylene was not so rapid as the rate of increase brought about by the original application. The percentage increase due to ethylene cannot be calculated accurately from the data given, but it is apparent that the increase due to ethylene is of the order of 200 per cent. Lot 849 showed an increase of about 260 per cent.

TABLE I

EFFECT OF ETHYLENE (1:1000) UPON RESPIRATION OF LEMONS

LOT NO.*	TREATMENT	CARBON DIOXIDE (MG.) RESPIRED†					
		Start	First day	Second day	Fourth day	Sixth day	Ninth day
821.......	Ethylene added at start, discontinuing at end of second day	8.0	21.5	29.7	23.0	20.5	....
832.......	Ethylene added at start, continued throughout	8.2	15.9	21.2	31.6	31.0	30.0
849.......	Ethylene added end of second day	6.8	8.2	9.7	26.7	29.7	....
841.......	Check; no ethylene	7.7	12.0	8.7	9.0	8.0	12.9

* These numbers also represent the weight in grams of the sample used.

† Milligrams per hour per kilogram of fruit.

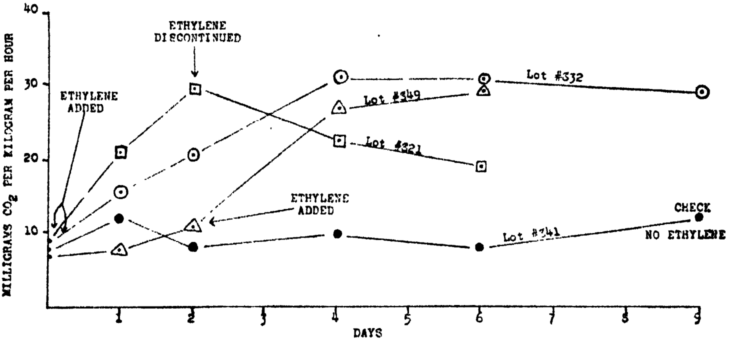

FIG. 1

The lot treated with ethylene throughout the experiment (no. 832) began to show yellowing about the third day. At this time also the "buttons," because of growth of the cells below, were raised slightly and could be pushed off by pressure with the thumb. Full yellow color had developed by about the seventh day. The untreated fruit remained green and the buttons were firmly attached. These results are in accordance with numerous previous experiments on the effect of ethylene on the coloration of green lemons.

Effect of different concentrations of ethylene

In this experiment concentrations of one part in one thousand, one part in ten thousand, one part in one hundred thousand, and one part in one million were used. To obtain such concentrations, a Florence flask with a capacity of 1.5 liters containing outdoor air was provided with a rubber stopper and glass tube inlet. A few cubic centimeters of mercury was added to form a seal when the bottle was inverted. Into this bottle enough ethylene was pushed over from a gas burette to make a concentration of 10 per cent ethylene. From this as a starting point, concentrations of 1, 0.1, and 0.01 per cent ethylene were made by dilution, using similar flasks as containers. Ethylene from such flasks was added to the desiccators containing the fruit to make the desired concentrations in the atmosphere surrounding the fruit. The data are tabulated in table II and are shown graphically in fig. 2.

The fruit used in this experiment had a higher respiration rate and showed greater variability than that used for the first experiment. In the case of each concentration tried, however, application of ethylene resulted in an increase in respiration (fig. 2). The increases ranged from about 100 to about 240 per cent. Many more data are needed to permit an accurate statement of the percentage increase. There are indications that some samples of fruit are more sensitive than others to ethylene and give greater responses. While with the lot of fruit used in this experiment considerable variations on different days were found with untreated fruit, the coincidence in point of time between the application of ethylene and the rise in respiration is proof that the rise is due to the ethylene.

While all the concentrations used caused an increase in respiration, 1:1,000,000 was less effective than stronger concentrations,

TABLE II

EFFECT OF DIFFERENT CONCENTRATIONS OF ETHYLENE UPON RESPIRATION OF LEMONS

Lot no.*	Treatment	Carbon dioxide (mg.) respired†					
		First day	Second day	Third day	Fourth day	Sixth day	Eighth day
736.......	Ethylene 1:1000 added at end of third day, continued throughout	13.8	....	9.7	26.1	31.7	41.0
728.......	Ethylene 1:1,000,000 added end of fourth day, continued throughout	15.9	....	16.9	13.1	25.0	31.6
729.......	Ethylene 1:100,000 added end of third day, discontinued end of sixth day	20.1	....	16.5	17.4	42.8	28.9
716.......	Ethylene 1:10,000 added end of sixth day	20.1	....	20.5	11.4	17.2	54.0
763.......	Check; no ethylene	....	15.4	17.4	12.7	17.0	18.6

* These numbers also represent the weight in grams of the sample used.

† Milligrams per hour per kilogram of fruit.

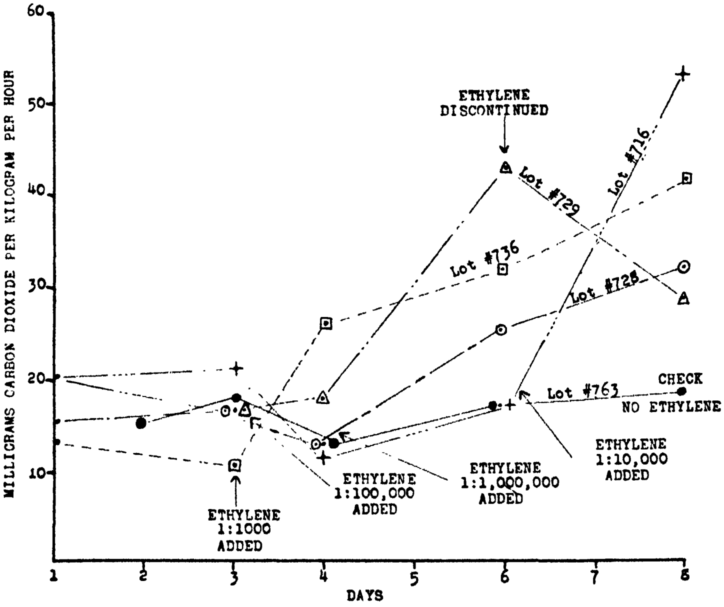

FIG. 2

indicating that the limit of dilution was being approached. There are also indications (fig. 2) that 1:1000 was too strong for best results, and that concentrations of 1:10,000 and 1:100,000 had a greater stimulating action. A larger number of experiments would be necessary to decide this point conclusively, however.

Discussion

No previous work appears to have been done on the effect of ethylene on the respiration of lemons; in fact, only one reference relating to the effect of ethylene upon the respiration of any plant was found. HARVEY (3), working with sweet pea seedlings at ethylene concentrations of 0.0002 per cent (by volume), found a depression in carbon dioxide output in every case but one. An essential difference in these two experiments should be noted. The sweet pea seedlings were undoubtedly in a state of active growth, while the lemons had practically completed their growth and were in a relatively dormant condition; hence the factor of toxicity should operate more strongly upon the sweet pea seedlings than upon the lemons. Thus a difference in the behavior of the two might be expected.

The exceptional case found by HARVEY is interesting in this connection. With the shortest period of exposure to ethylene, the 3-hour period, he found that the ethylene treated lot showed an increased respiration over the check lot. A stimulative action producing an increase in carbon dioxide production shortly after the application of the gas, before the anaesthetic effects could become apparent, would be in agreement with the results obtained by IRVING (4). Investigating the effect of chloroform vapor upon the respiration of barley and cherry laurel leaves, IRVING found that the response depended upon the concentration used. Low concentrations gave increases that were maintained during the entire experimental period. High concentrations depressed the respiration in every case. Medium concentrations caused an "initial outburst" of carbon dioxide, an expression that was justified by the data. Thus in one case he found that the respiration was increased 200 per cent (trebling the rate) after one hour's exposure. The percentage increase in this case is of the same order as that found in the experiments with lemons here reported.

IRVING's data show that the high initial rates with these medium concentrations were not maintained, but were later followed by a decrease. With lemons, however, the rate produced with the strongest concentration (1:1000) was maintained fairly well for five days, with only a slight decrease (lot 832, fig. 1); hence there is evidence of only low toxicity, if the toxicity may be judged by fall in rate of respiration. The concentrations used appear to be stimulative rather than depressive.

Summary

1. Ethylene at concentrations of one part in one thousand of air, one part in ten thousand, one part in one hundred thousand, and one part in one million increased the respiration of green lemons. The effect appeared to be greatest at the intermediate concentrations, although more work to establish the concentration yielding the maximum effect is necessary.

2. The increase in carbon dioxide output ranged from about 100 per cent to about 250 per cent.

3. After the respiration of a given lot of lemons had been increased by the use of ethylene, a discontinuance of the ethylene applications led to a decrease.

4. Yellowing of the ethylene treated fruit became visible about the third or fourth day, and full yellow color was developed in six to ten days. Untreated fruit remained green during the same period of time.

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GROWTH OF PLANTS IN ARTIFICIAL LIGHT

II. INTENSITIES OF CONTINUOUS LIGHT REQUIRED FOR BLOOMING

ESTEN HENDRICKS AND R. B. HARVEY

(WITH ONE FIGURE)

The intensity of light at which plants are grown is related to the rate of growth. In continuous illumination there is a constant rate of synthesis of carbohydrates, and the rate of growth is uniform. Under such conditions there is no periodic daily fluctuation in carbohydrate content or in growth rate, such as occurs in plants which are grown in sunlight. By the use of Mazda lamps as the source, the intensity of light can be kept fairly constant, so that one can determine definitely what intensity is required for maintenance of the plant or for flower production. KRAUS and KRAYBILL[1] have shown that fruiting is dependent upon a certain ratio of carbohydrates to other nutrient constituents; so one would expect blooming to be dependent upon a suitable intensity of light.

From common experience it is observed that plants vary in their requirements of light intensity. It was the object of this study to determine what these requirements are, when temperature is kept constant and other environmental factors are held as nearly uniform as possible. Obviously, the light intensity required for the continued growth of a plant must be such that the assimilation will at least overbalance the loss by respiration.

Plants were grown in the rooms described in a previous paper.[2] They were planted in good soil and watered with Knop's nutrient solution occasionally, so that there was no deficiency of mineral nutrients. The light intensity was varied by placing the plants at different distances from the lamps. The Macbeth illuminometer was used to measure light intensity, reading directly in foot candles.

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[1] KRAUS, E. J., and KRAYBILL, H. R., Vegetation and reproduction with special reference to the tomato. Oregon Agric. Coll. Exp. Sta. Bull. 149. 1918.

[2] HARVEY, R. B., Growth of plants in artificial light. BOT. GAZ. 74:447–451. 1922.

Where there was a considerable increase in height of the plants as they grew, the intensities are given over the range covered. Several measurements of light intensity were taken during the period of growth. The plants were subjected to a range of intensity from 50 to 10,632 foot candles. Only the intensities at which the plants bloomed are recorded in table I. No doubt in the higher intensities some varieties were subjected to excessive temperatures, owing to the light absorption by the leaves. Since in most cases the plants were grown from seed, they assimilated practically all of their dry weight in the artificial light.

Amaranthus retroflexus and *Chenopodium album* bloomed at notably high light intensities, but were stockier than those plants which bloomed at lower intensity. Evidently these plants have a high tolerance for light. Strawberries bore fruit at 1500–2000 foot candles, but did not bloom at 500 foot candles. Plants grown at intensities below their proper range tend to be spindling, and are likely to become diseased. *Nicotiana tabacum* grew well at low intensity and bloomed over a great range of intensity. *Fagopyrum* and *Tropaeolum* seem adapted to growth at low intensities, in fact at intensities at which a great many plants cannot maintain themselves.

The four-o-clock produced abundant flowers at 1350 foot candles, but only by violent fluctuations in temperature or moisture could the flowers be opened in continuous light. When the temperature and soil moisture were kept uniform, the flowers remained unopened although fully formed, until an abscission layer formed and they dropped off. After a number of flowers were fully formed and past the time at which they should have opened, the lights were turned off for an hour. Then ten flowers promptly opened. Squash produced staminate flowers abundantly at 476 foot candles, but only a few pistillate flowers.

On December 27 some Easter lily bulbs were just sprouting. The plants shown in fig. 1 were photographed on February 27. The lily on the left was grown in continuous artificial light at 20° C., the one on the right was grown in the greenhouse at a fluctuating temperature which averaged above 20° C. In artificial light Easter lilies can be speeded up to bloom within two months after sprouting. The plants in the greenhouse did not bloom until a month later.

TABLE I

INTENSITIES OF LIGHT AT WHICH PLANTS BLOOMED AND PRODUCED SEED

Plant	Temperature ° C.	Light intensity in foot candles
Boltonia asteroides	25	950
Cucurbita moschata*	25	550
Pisum sativum	14	400
Silene latifola	25	800
Lactuca sativa	25	900–1350
Erigeron canadensis	25	1040
Melilotus alba	14	1180
Phaseolus vulgaris	25	850
Stellaria media	25	920
Avena sativa	14	950
Hordeum vulgare	14	650–800
Secale cereale	14	600–1200
Triticum vulgare (Kota)	14	650–800
Triticum vulgare (Winter)	14	650–800
Triticum vulgare (Bluestem)	14	650–800
Triticum durum (Monad)	14	650–800
Raphanus sativus	14	1400
Trifolium pratense	14	1400
Melilotus officinalis	14	1400
Viola tricolor*	25	1000
Tropaeolum minus	20	250–450
Oxybaphus nyctagineus	20	1350
Cucurbita moschata	20	250–500
Linum usitatissimum	20	500–1050
Nicotiana tabacum	20–25	800–10,032
Zea Mays	25	500–3000
Euphorbia splendens	20	800
Lilium longifolium	20	1200
Salvia sp	20	300
Pelargonium sp	20	350–550
Trifolium hybridum	20	400–600
Chenopodium album	20	500–10,632
Amaranthus retroflexus	20	500–10,632
Solanum nigrum	20	750
Phaseolus vulgaris	20	300–550
Cannabis sativa	20	300–450
Dianthus barbatus	20	650
Fragaria sp	20	1500–2007
Cucumis melo	20	500–2007
Fagopyrum esculentum	20	300–400
Hibiscus trionum	25	600–900
Solanum tuberosum*	25	450–500
Curcurbita Pepo*	25	500–750
Aster sp	25	500–2007
Viola sp	25	....

* Bloomed but no seed set.

Evidently the quantity of material assimilated by the leaves serves to increase the growth rate over that of plants with day and night exposure. In table II is given the composition of the lily leaves collected at 3:00 P.M., when the plants in daylight should show a maximum of starch. The greater quantity of carbohydrates in the plants grown in artificial light is quite marked.

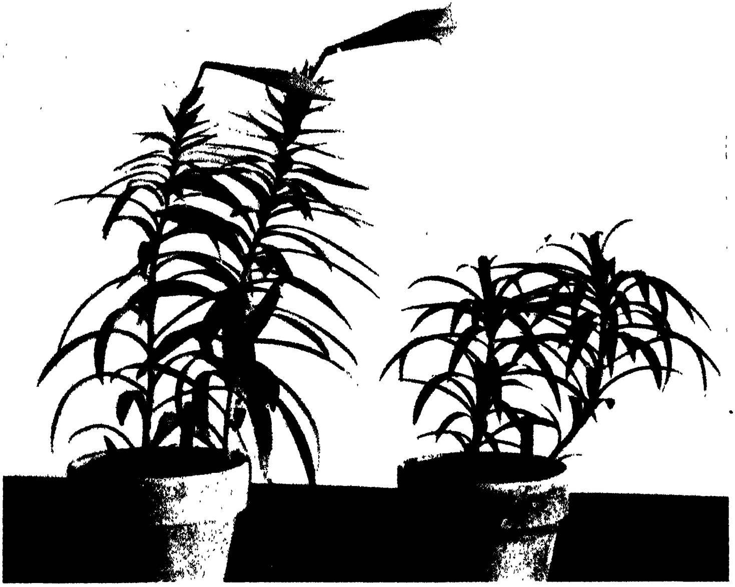

FIG. 1.—Plant on right grown in daylight; plant on left grown in continuous artificial light.

TABLE II

CARBOHYDRATES OF EASTER LILY*

SAMPLE†	DRY WEIGHT PERCENTAGE	SUGARS			STARCH PERCENTAGE	PENTOSAN PERCENTAGE	TOTAL NITROGEN PERCENTAGE
		Reducing percentage	Sucrose percentage	Total percentage			
Daylight........	10.91	3.23	6.22	9.45	2.35	6.96	3.34
Artificial light....	9.78	10.51	2.92	13.43	6.41	8.89	2.34

* The writers are indebted to Mr. F. R. DAVISON for these analyses.

† Both samples picked at 3:00 P.M., March 20, 1923; samples run in duplicate or triplicate.

Conclusions

1. A number of plants have been subjected to continuous artificial illumination and determinations made of the light intensities at which blooming occurred.

2. Plants were found which showed growth and blooming at a great range of intensities, while others were found to bloom only at a limited range.

3. A speeding up of the time of blooming in Easter lilies was correlated with an increase in the carbohydrate content of the leaves when grown in continuous artificial light.

University Farm
St. Paul, Minn.

SMOKE AND SOIL ACIDITY

ARTHUR PIERSON KELLEY

(WITH TWO FIGURES)

The southern part of the "peninsula" upon which the city of Philadelphia is built is a slightly undulating plain with an elevation varying from sea level to thirty feet. For many years civic growth was in other directions, while this southern area remained as a region of small truck farms, but houses and factories are now encroaching upon it. With this industrial development have come repeated complaints from the truck farmers that their crops were damaged by various emanations from the nearby factory stacks. At the suggestion of Professor RODNEY H. TRUE, to whom the writer is much indebted for advice during the prosecution of the work, a soil survey of the region was undertaken to determine whether or not the agricultural soils of the region were acid, and if acidity were present, whether its distribution could be correlated with fume production from the industrial plants. If these plants were giving off considerable quantities of acid fumes, it seemed probable that these would have accumulated in the soils of the region in such quantities as could be measured by the methods here used.

Investigation

SOIL.—The area investigated, including about three square miles, is part of the coastal plain, and is covered by an alluvial deposit called Sassafras soil,[1] formed from weathering of unconsolidated deposits of late geological age. Sassafras sandy loam consists of a light brown or yellowish gritty sandy loam becoming heavier with depth. Both soil and subsoil contain mica flakes and fine gravel. In places the latter is found in considerable quantities. Almost surrounded by towns, with rivers on three sides used by many steamers, the land intersected by railroads and dotted with factories, it would seem that the region might be subject to smoke injury.

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[1] SHAW, C. F., Soils of Pennsylvania. Penn. Agric. Exp. Sta. Bull. 132. 1914.

ACIDITY OBSERVED.—Work was done in this region during the months of July and August, 1922, on both cultivated and uncultivated land in different parts of "The Neck" at varying distances from the most conspicuous plants. Borings were made with a soil auger to levels from 15 to 90 cm., and the samples thus obtained were carried immediately to the laboratory in corked test-tubes. Here they were mixed with distilled water in 1:3 proportion and tested with Clark and Lubs indicators by the use of a comparator and standards. The results of these tests are shown in table I. It will be seen that the soils are acid, but that the geographical evidence seems to indicate that the acidity is not to be correlated with any one industrial plant (fig. 1). Being almost encircled with smoke sources, the atmosphere of "The Neck" would be smoky no matter what the direction of the wind. Moreover, soil

TABLE I

P_H VALUES

TEST BORINGS NO.	DEPTH OF SAMPLES IN CM.						
	Surface	15	30	45	60	75	90
2261.......	5.6	6.0	6.0	5.8	5.8	6.1	6.1
2268.......	6.0	5.7	6.1	6.1	6.4	6.5	6.5
2275.......	6.2	5.7	5.6	5.8	5.8	5.9	6.0
2282.......	6.2	6.0	6.1	6.2	6.2	6.4	....
2288.......	6.2	5.6	5.4	5.7	5.7	5.7	....
2221.......	5.6	5.6	5.8	5.8	....	....	....
2228.......	5.8	5.6	5.6	....	....	....	....
2000.......	5.0	5.1	5.3	5.5	5.8	5.8	6.0
2007.......	5.5	5.6	5.6	5.6	5.6	5.8	6.0
2014.......	5.6	....	....	....	....	....	....
2021.......	5.6	5.6	5.7	5.6	....	....	....
2028.......	5.6	5.6	5.6	....	....	....	....
2035.......	5.8	5.6	5.1	4.9	5.7	5.8	5.8
2042.......	6.1	5.6	5.4	5.6	6.0	6.1	6.3
2091.......	5.4	5.8	6.4	6.8	6.8	6.8	6.6
2305.......	6.0	5.7	5.4	5.6	5.8	5.4	5.4
2312.......	5.8	6.0	6.8	6.8	7.0	7.0	7.0
2319.......	6.0	5.6	5.6	5.8	5.8	6.1	5.9
2326.......	6.0	6.1	6.1	6.3	6.1	6.4	6.7
2333.......	6.0	6.0	5.6	5.8	5.8	5.4	....
2340.......	5.4	5.4	5.6	....	5.8	5.9	5.9
2347.......	5.4	5.6	6.0	6.0	....	5.4	5.8
2354.......	5.6	5.8	5.8	6.0	6.0	6.0	6.0
Average..	5.75	5.69	5.75	5.84	6.35	6.47	6.13

immediately adjoining the most important works was not acid. Similar results have been found by Just and Heine.[2]

Smoke and acidity.—A question now arose as to whether acidity found in the soil was caused by smoke, or whether it was a natural condition of Sassafras soil. To determine this an investigation has been made of a comparable area of Sassafras loam at Monmouth Junction, New Jersey, where the atmosphere, while not entirely free from smoke, was much more nearly so than in South Philadelphia. The results are given in table II. In general a sandy soil may be normally acid, as indicated by a DeKalb area in the North Valley Hills of Chester County, Pennsylvania. A more or less similar curve on the acid side is again exhibited (fig. 2).

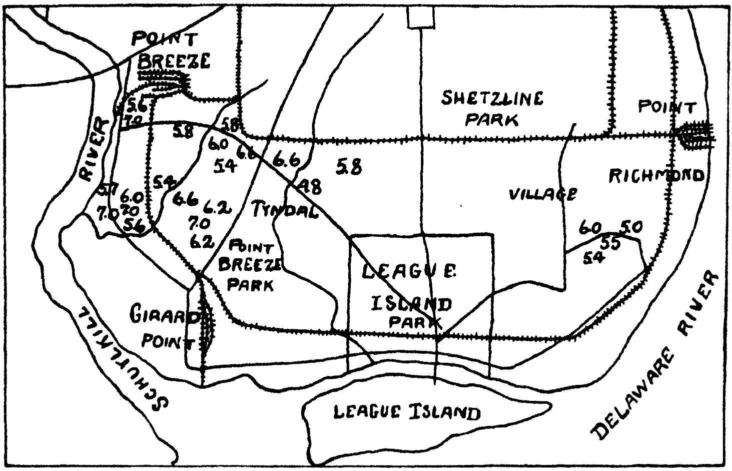

Fig. 1.—Map of South Philadelphia; figures indicate P_H values

Soil acidity and sulphur.—Tests were made to discover the sulphur dioxide content of soils from various places near the Point Breeze works. They were carried out according to the method given by Haywood,[3] and the results are given in table III, together with some obtained for soils from Monmouth Junction, New Jersey, and from Paoli, Pennsylvania. Waters flowing out of the region apparently are contaminated by sewage. Two natural streams remain, and there are a number of drainage canals, especially in the lower lying eastern section, the waters of which in all cases observed were over P_H 7.0. Dew on plants at Remerten's Nurseries at least on one day was about P_H 7.0, while at other times and in

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[2] Just, L., and Heine, H., Zur beurteilung von vegetationschaeden durch saure gase. Landw. Versuchs-Sta. 36:135–158. 1889.

[3] Haywood, J. K., Injury to vegetation by smelter fumes. U.S. Dept. Agric. Bur. Chem. Bull. 113. 1908.

TABLE II

P_H VALUES

TEST BORINGS NO.	DEPTH OF SAMPLES IN CM.				
	Surface	15	30	45	60
1178..........	6.0	6.0	5.8	....	....
1183..........	6.2	5.7	5.6	5.6	....
1187..........	5.7	5.7	5.3	5.3	5.3
1192..........	5.4	5.8	6.0	6.1	6.1
1202..........	4.4	4.5	4.6	5.2	5.0
1499..........	6.0	5.8	6.0	6.0	6.0
1504..........	5.8	5.9	6.0	6.6	6.6
1509..........	6.0	5.6	5.8	6.0	6.2
1514..........	6.4	6.1	6.2	6.4	6.4
1520..........	5.8	6.0	6.2	6.4	6.0
1525..........	6.3	6.5	6.8	7.0	6.4
1530..........	6.2	6.1	5.8	5.4	5.6
1535..........	6.0	5.8	6.0	6.4	6.4
1547..........	5.4	5.2	5.8	5.6	5.8
Average.....	5.82	5.75	5.83	6.00	5.98

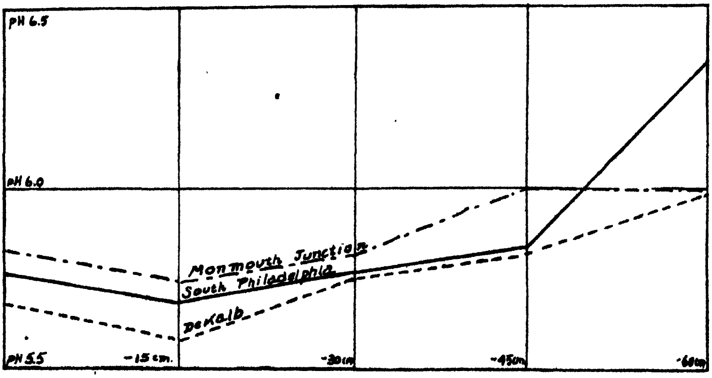

FIG. 2.—Relation of soil depth to soil acidity; figures indicate P_H values

other places the P_H was less, to as low as 5.6. Even in an area subject to smoke influence soil may not of necessity be acid, nor crops seriously affected. A half-acre garden on Manor loam at Paoli, Pennsylvania, almost constantly under an atmosphere laden with fumes blown over from the railroad yards, was kept under close observation. With constant cultivation during the growing season and proper use of fertilizers, the soil is kept at a P_H about 7.0, although Manor loam is normally at a P_H of 6.4.

TABLE III

Test borings no.	Location	Percentage SO_3, as $BaSo_4$	P_H
2	Grounds of Atlantic refinery	1.37	7.0
6	Overgrown field, Girard Point	1.33	5.6
7	Marsh, Girard Point	1.32	5.4
8	Hay field, Girard Point	1.38	6.6
20	Cultivated field, Girard Point	2.91	6.6
28	Garden, Monmouth Junction, N.J.	0.74	6.1
38	Field, 1 mile from railroad, Paoli	2.44	6.4
40	Garden, cultivated, Paoli	3.16	7.2
42	Garden, uncultivated, Paoli	2.23	6.8

Summary

In the trucking section of South Philadelphia the air is often heavily loaded with combustion products from great industrial plants, railroad engines, and other sources. The soils of this region, consisting of Sassafras loam, are somewhat acid but not more so than another area of the same soil type at Monmouth Junction, New Jersey, where there is little smoke. Other sandy soils, of DeKalb areas, have shown approximately the same P_H values. The geographical distribution of acidity in South Philadelphia apparently is not correlated with the location of the smoke producing plants. With better cultural methods, cultivated lands in another type of soil have been kept near neutrality.

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BRIEFER ARTICLES

THREE SETS OF MEGASPORANGIATE CONES PER YEAR IN PINUS[1]

(WITH TWO FIGURES)

Professor W. E. DAVIS called my attention during the spring of 1923 to a certain specimen of *Pinus Banksiana*, which he had been passing every few days, as exhibiting something very peculiar to him. Investigation showed that this tree was producing two and three sets of "yearly growths" during this spring, each of which had a bare space at the base where the microsporangiate cones normally occur. The leaves were as usual, and near the tip of each "year's growth" there were one or two (and in one case three) megasporangiate cones. At least four branches were found in which there were present three complete sets of megasporangiate cones, one set for each "year's growth." Several were found in which they were in the first and second or first and third "year's growths." When observed in the middle of the growing period, the different growths were progressively smaller in diameter, and had progressively smaller leaves, but the megasporangiate cones were practically all of the same size. Several small side branches produced the microsporangiate cones. In all such cases only the normal year's growth took place.

The tree was medium sized, a little under three meters in height, and had been transplanted to this spot at least four years previously. The growth was very vigorous on the branches as well as on the leaders. The peculiar growth took place in 1922 also, but more sparingly. Previous to that it apparently had not taken place.

Inspection of the cut ends showed the proper number of annual rings of normal size in accordance with the actual age, but closer examination with a compound microscope disclosed the fact that in at least some of the cases there were faint rings within the conspicuous annual rings, the same in number as that of the apparent "year's growths." During the period of growth in length one could tell the "year's growths," both by the thickness and by the fact that the newest growth was green throughout, but in the older "year's growths" of this year one or two layers of whitish

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[1] Contribution from the Botanical Laboratory of Kansas State Agricultural College, no. 205.

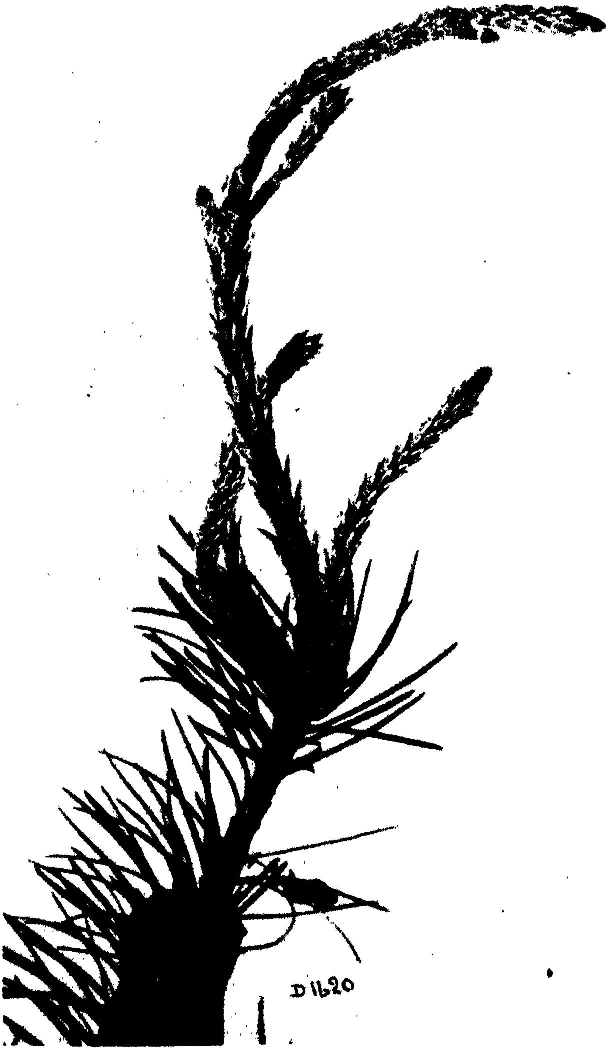

FIG. 1.—End of branch of *Pinus Banksiana* Lamb, showing three sets of megasporangiate cones produced in 1923.

wood were evident. As the twigs increase in age the faint rings usually vanish. Consequently, later in the season or in later years the growths can be told apart positively only so long as they are of a size, or retain their cones, or have similar aged branches.

C. S. SARGENT, in his *Manual of the trees of North America*, in describing the cones, states for this pine: "Often with two clusters produced on the same shoot." This has been noticed previously on *Pinus Banksiana* in this vicinity, but at the present time this case is the best one that has been found, in which three sets of megasporangiate cones have been produced in a single year. At least five other trees of this species have been found which produced three growths in a year, although but sparingly. Both 1922 and 1923 have been prominent in this respect, although the seasons were quite different, that of 1922 being an early spring, while the spring of 1923 was very late. The summers were similar, although that of 1922 was very much hotter.—FRANK C. GATES, *Kansas State Agricultural College, Manhattan, Kansas.*

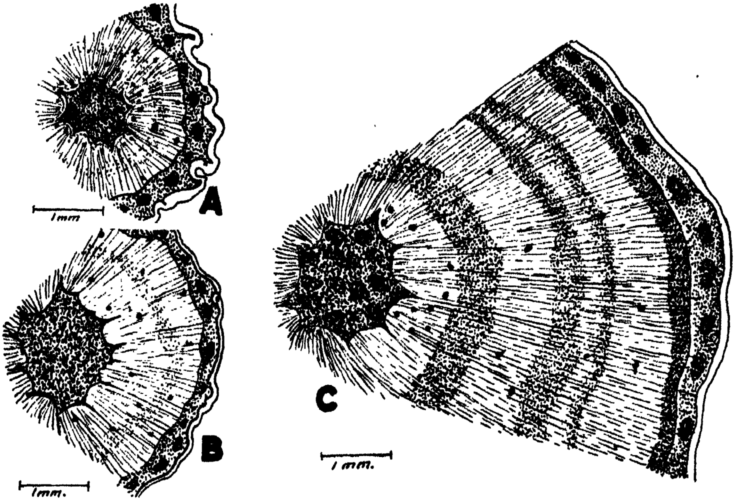

FIG. 2.—Diagrammatic sketches of cross-sections of twigs of *Pinus Banksiana* of different ages, collected in the fall: *A*, youngest or last of three growths produced in 1923; *B*, oldest or earliest of three growths produced in 1923; *C*, three-year-old twig, latest growth as shown by cones; sketches by S. FRED PRINCE.

LACTOPHENOL

Investigators and teachers of natural science have need for a single solution in which freehand sections and small specimens of plants and animals can be fixed, stained, preserved, and permanently mounted. Lactophenol, a solution which partly accomplishes this purpose, was called to my attention by Dr. J. J. Davis of the University of Wisconsin. After using lactophenol for several years for various purposes, it seems desirable to call the attention of teachers and investigators to its merits.

A translation from Strasburger-Koernicke, *Botanisches Praktikum*, Fünfte Auflage, 767, 1913, follows:

Lactophenol: Lactophenol may be used alone or with copper solutions as a preservative material for mosses and algae. Lactophenol may be prepared:

1. Carbolic acid crystals	(c.p.)	20 gm.
2. Lactic acid	(sp. gr. 1.21)	20 gm.
3. Glycerine	(sp. gr. 1.25)	40 gm.
4. Water	(distilled)	20 gm.

This solution combines the clearing qualities of carbolic acid with the softening properties of lactic acid. It is very good for herbarium materials, especially when warmed with a 10 per cent lactophenol solution and transferred to pure lactophenol. For preserving desmids, Palmellae, and filamentous algae, place 5 gm. lactophenol with 95 cc. distilled water, then add 0.2 gm. copper acetate. This same solution, ten times more concentrated, is recommended for use on excursions. Place 5–10 per cent of the concentrated solution in water containing algae, which it fixes so that it can be stored for a long time. The chlorophyll holds its color in this solution. Glycerine gelatine may be employed as a mounting medium. Soak 8 gm. of gelatine in 44 cc. distilled water, then add 30 gm. glycerine, cook in a water bath, filter, and add 10 gm. lactophenol thereto. The preparations become as transparent as in Canada balsam, and are well preserved. Instead of lactophenol, one can add a concentrated lactophenol-copper solution to glycerine jelly. Green and blue pigment are well preserved.

Lactophenol gum as a mounting medium: dissolve 38 gm. very pure gum arabic in 50 cc. fresh distilled water, add 5 gm. glucose, 6 gm. lactophenol, and filter the solution through glass wool. It is used cold and dries very quickly.

In mixing the solutions composing lactophenol as described in this formula, the uncorked bottle of carbolic acid crystals should be set in a dish of water, and heat applied slowly until the crystals are in solution. The remainder of the process in preparing the formula needs no explanation.

An acid stain may be added to lactophenol when either differential staining or preservation of colors is desired. Stains used with best success by the writer are saüregrün, acid fuchsin, and acid carmine. A stock solution of green lactophenol was prepared by adding 0.10 gm. of Grubler's säuregrün to 100 cc. lactophenol. This stock solution may be used undiluted, or diluted by placing 10 cc. of green lactophenol into each of three pipette bottles containing 10 cc., 20 cc., and 40 cc. of clear lactophenol respectively. Experience showed different materials required different intensities of the colored stock solutions, and by this method three good working colored solutions were at hand. Generally, colored solutions of lactophenol were prepared by adding the stain to lactophenol until the solution gave a deep color when examined by transmitted light. Permanent sections should be mounted in a deep colored lactophenol solution, since the stain fades when exposed to bright light.

When microscopic slides containing sections mounted in lactophenol were stored, evaporation took place. If more lactophenol was flowed under the cover glass and the process repeated, evaporation was reduced to a minimum. If the slides and covers were cleaned and ringed with a good cement, however, evaporation of the lactophenol was entirely prevented and the mounted materials remained in an excellent state of preservation. While lactophenol has its limitations, when once used in the laboratory it will meet a long felt need.—W. H. Davis, *Massachusetts Agricultural College, Amherst, Mass.*

CURRENT LITERATURE

NOTES FOR STUDENTS

Photosynthesis and respiration.—In their studies of the physiological processes of green leaves, BROWN and ESCOMBE showed that leaves utilize in photosynthetic activity about 0.66 per cent of the radiant energy they receive. Furthermore, they calculated that if the leaf could utilize all the energy of that part of the spectrum in which chlorophyll absorbs most strongly, the efficiency of energy transformation would not exceed 6 per cent, since those regions of the spectrum contain only about 6 per cent of the total solar energy. WARBURG and NEGELEIN[1] have obtained a theoretical efficiency of energy transformation of 70 per cent, using a narrow region of the spectrum, 645–570 $\mu\mu$, and an intensity about 1/1000 of normal daylight. The light was thrown into cultures of *Chlorella* of such thickness as to give total absorption, and the energy transformation was calculated from the amount of O_2 liberated, corrections being made for respiratory use of oxygen. The observed efficiency was about 50 per cent, from which was calculated the initial efficiency for the first increment of light above zero. This extrapolated efficiency was 70 per cent.

Cells grown in weak light previous to use in experiments gave a higher efficiency than cells grown in strong light. This is explained on the basis of an adsorption theory which has been developed by WARBURG[2] with reference to photosynthesis and respiration. The photosynthetic reactions are considered to be adsorption reactions, and in brightly illuminated cells there is more free glucose. This glucose is adsorbed on the phase surfaces to a degree that retards the adsorption of the substances reacting in synthesis. As there are always some adsorbable substances present in the cells besides the reactive ones, and as the energy adsorbed by the chlorophyll is available for photosynthesis only during 10^{-8} seconds, it is evident that perfect efficiency is impossible. Evidence resulting from the application of the quantum theory to these problems lends support to such theories as have been put forward by WILLSTÄTTER and DIXON, who believe that the first reactions of photosynthesis involve intramolecular rearrangements either in H_2CO_3 or in the chlorophyll itself. In order to secure the requisite energy, one molecule of H_2CO_3 must interact with three molecules of chlorophyll.

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[1] WARBURG, O., and NEGELEIN, E., Über den Energieumsatz bei der Kohlensäureassimilation. Arch. Neerl. Physiol. de l'homme et des animaux. 7:415–430. 1922.

[2] WARBURG, O., Über Oberflächenreaktionen in lebenden Zellen. Zeit. Elektrochem. 28:70–75. 1922.

The importance of adsorption in respiration has been illustrated by means of a model cell, in the second paper cited. This consists of a solution of amino acids containing powdered charcoal. On passage of a current of air through the liquid, oxidation of the amino acids occurs at a rate equal to the respiration of an equal mass of living tissue. The charcoal, by adsorbing the interacting amino acids and oxygen on its surface, causes the reaction to be greatly hastened. Similarly, the great acceleration within the cell of processes that are infinitely slow outside it, WARBURG believes to be due to the adsorption of the reactive substances on the surfaces of the solid constituents of the cell.

The effects of anaesthetics upon living cells, and upon this model cell of powdered charcoal, are very similar. Thus, for the same degree of depression of respiration or photosynthesis, homologous series of anaesthetics show a progressive anaesthetic efficiency from member to member, the highest member of a series being from 100 to 1000 times as effective as the first member. On calculating the surface area covered by the anaesthetics at equal depression of the respiratory rate, it is found to be a constant. This constitutes strong evidence of the correctness of the theory. Moreover, these anaesthetics affect the oxidation of amino acids in the charcoal-containing model cell in exactly the same way as they affect the life processes.

The anomalous behavior of HCN which, although adsorbed quite weakly, is effective in suppressing respiration in very low concentrations, leads to the hypothesis that the surfaces of the solid cell constituents are mosaics of Fe-containing and Fe-free regions, the latter greatly predominating. Respiration and photosynthesis are supposed to be catalysed on the Fe-containing regions only, although the reacting respiratory and synthetic substances are adsorbed equally over the whole surface. The hydrocyanic acid, because of its strong affinity for the heavy metals, replaces the reactive substances only on the iron-containing areas, and thus little is needed to depress respiration, whereas anaesthetics replace them over the entire surface equally, and much is required unless adsorbed strongly. The rôle assigned to iron here is quite in accord with that put forward by MOORE some years ago. Such careful fundamental theoretical work as this is highly inspirational.—H. S. WOLFE.

Plant tumors and animal cancer.—LEVIN and LEVINE,[3] in a series of articles designed primarily for medical readers, endeavor to shed light upon certain aspects of the cancer problem through studies of similar growths in plants. They take exception to the view that cancer and crown gall are practically identical biologically, but consider that there are many points of analogy

[3] LEVIN, I., and LEVINE, M., Malignancy of the crown gall and its analogy to animal cancer. Jour. Cancer Research 5:243–260. 1920.

———, The rôle of neoplasia in parasitic diseases of plants. Jour. Cancer Research 7:171–178. 1922.

———, The action of buried tubes of radium emanation on neoplasias in plants. Jour. Cancer Research 7:163–170. 1922.

between the two, and that since a plant is much less complex in its organization than an animal, neoplasias in plant tissues offer favorable material for studies of cell proliferation. It is claimed that crown gall in plants is commonly analogous to benign tumors in animals, and that malignant crown gall, while analogous to cancer, is relatively rare and is not due directly to infection by *Bacterium tumefaciens*, but rather represents the result of the action of an unknown secondary stimulus, which has altered the limited growth, begun for purposes of protection against bacterial invasion, into a condition of limitless proliferation.

It has long been noted that in club root caused by *Plasmodiphora brassicae* the large cells containing the parasite are always surrounded by layers of uninfected small cells. A similar condition occurs in potato wart lesions. The theory is advanced that these smaller cells are immune to the parasite and tend to form a protective barrier against its further encroachments.

The treatment of animal tumors by the insertion of capillary glass tubes filled with radium emanation demands a more complete knowledge of the mechanism of the radium action. For this investigation, also, plant tissues suggested themselves as favorable material. The effect of the radium emanation on both normal and diseased tissue was studied. The radium tubes were inserted as in animal tissues, and in every case empty glass tubes of similar size and shape were inserted into corresponding tissues as checks. Where normal tissue was used the radium emanation invariably induced necrosis, usually slight, but varying in degree according to the amount of radium emanation in the tube, the length of the exposure and the kind and age of the tissue. No necrosis was observed in the checks. Inoculation of *Pelargonium* stems with the crown gall organism, followed immediately by the insertion of the radium tubes, resulted in the formation of a black necrotic area around the tubes, but no galls were produced. Inoculated stems into which the empty tubes were inserted developed typical crown galls. If the introduction of the radium tube was delayed until the gall had developed, there was death of cells and necrosis in the region immediately adjacent to the tube, but cells beyond were morphologically unaffected. Earlier work is cited, however, suggesting that such cells have been so influenced by the gamma rays as to have had their proliferating power inhibited. Similar results were secured on club roots artificially produced in cabbage and kohlrabi by inoculation with *Plasmodiophora brassicae*.—G. W. MARTIN.

Shape of parenchyma cells.—If college students in botany were asked to describe the 3-dimensional shape of a typical plant cell, their replies would probably include the range of opinion that it is "six-sided" (cubical?) to irregularly polyhedral; a few might state that its shape is that which a plastic sphere would assume if surrounded by other similar spheres all of which touched; some might describe this "flattened sphere" as a rhombic dodecahedron. The latter is indeed the opinion that dates back to KIESER and BUFFON, who arrived at this conception by the analogy of a stack of cannon balls, or of dry peas which are made to swell in a closed chamber. LEWIS,[4] by means of models reconstructed from serial sections of elder pith, has studied the form of the typical cells of one mature plant tissue. KELVIN had demonstrated mathematically in 1887 that of all bodies which may be combined to fill space without interstices a tetrakaidecahedron, or figure having 14 surfaces, certain of which are slightly curved, rivaled the rhombic dodecahedron of KIESER in inclosing "the greatest space with the least extent of surface." By actual count of the numbers of contacts which each cell of elder pith has with those surrounding it, LEWIS has determined that these cells actually are approximately 14-sided, a mean number of 13.96 contacts having resulted from counts on 100 cells. More than half are 13, 14, or 15-hedra. "Cells with an excessive number of contacts are usually large, and of such form as to suggest that an expected division in some particular plane has failed to occur." The manner of restitution of the tetrakaidecahedron following cell division, which produces an 11-sided figure, whether the bisecting plane be vertical or transverse, was determined by a study of actual cell models.—F. WEISS.

Light and growth of plants.—Ever since the work of GARNER and ALLARD, showing a close relationship between the length of day and growth and reproduction in plants, plant physiologists have felt the need of a thorough study of the chemical conditions existing in plants exposed to light for different periods of time. A recent paper by NIGHTINGALE[5] does much to supply this need. Using mainly the tomato, but including several other plants, he exposed the plants to light for different lengths of time, and also varied the amount of nitrogen in the nutrient solution. He finds that in the tomato it is the carbohydrate-insoluble nitrogen ratio, rather than the carbohydrate-total nitrogen ratio that is significant in determining the type of growth. Strongly vegetative unfruitful plants were secured under conditions resulting in a high proportion of insoluble nitrogen to carbohydrates. These conditions were a short day and a good nitrate supply. If the nitrogen supply was kept up and the day lengthened, conditions were present for the formation of an abundance of insoluble nitrogen and also of carbohydrates. The result was strongly vegetative and fruitful plants. Conditions of long day and low nitrate supply resulted in a high proportion of carbohydrate to insoluble nitrogen and a weakly vegetative and unfruitful plants. Buckwheat, soy beans, radish, and *Salvia* seemed to require a certain duration of light as well as a supply of carbohydrates for the synthesis of nitrates to insoluble nitrogen. This was not true in the case of the tomato, at least within the limits of the light exposure tested.—S. V. EATON.

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[4] LEWIS, F. T., The typical shape of polyhedral cells in vegetable parenchyma and the restoration of that shape following cell division. Proc. Amer. Acad. Arts and Sci. **58**:537–552. 1923.

[5] NIGHTINGALE, G. T., Light in relation to the growth and chemical composition of some horticultural plants. Proc. Amer. Soc. Hort. Science 1922: 18–29.

Light penetration into seawater and plant distribution.—Using a Kunz photo-electric cell, SHELFORD and GAIL[6] have made a study of light penetration into seawater under different conditions. They find that in clear, calm weather the surface of the water shuts out 25 per cent of the sunlight. If the surface is rough, as high as 70 per cent may be shut out. When the surface of the water is smooth, about 60 per cent of the light penetrates to a depth of 1 m. The authors also studied the penetration of light of different wave lengths. Then this quantitative data is related to the distribution of vegetation. Most of the brown algae are found at a depth of 10 m., where the short waves of light are about 10 per cent and the red about 0.99 per cent of full sunlight. The red algae are found between 15 and 25 m., where the shorter wave lengths are between 10 and 2 per cent and the red between 0.99 and 0.0032 per cent of full sunlight. Thus in both cases, the energy for photosynthesis is mainly from the shorter wave lengths. The work has been done carefully and accurate quantitative data on at least one of the factors affecting plant distribution in the sea have been determined.—S. V. EATON.

Sand and water cultures.—In a series of carefully planned and conducted experiments with Marquis wheat, BAKKE and ERDMAN[7] studied the best proportions of the three salts KNO_3, $CaH_4(PO_4)_2$, and $MgSO_4$, for optimum growth in sand cultures as compared with these in water cultures. The data reported include records of transpiration, green and dry weights, temperature, evaporating power of air, radiant energy, and hours of sunshine. Pronounced changes in hydrogen ion concentration during the final 3.5 day period of growth (and hence presumably in earlier periods also) are shown to have occurred. The tendency to approach neutrality was greater in the sand than in the water cultures. No correlation could be shown between total yields and reaction values of the nutrient media, a result which might have been anticipated in the absence of buffered solutions. The water culture giving most favorable growth of top had 2/8 of its total osmotic concentration derived from KNO_3, 1/8 from $CaH_4(PO_4)_2$, and 5/8 from $MgSO_4$. With sand, best top growth was attained in a corresponding 3/8 KNO_3 solution, 3/8 $CaH_4(PO_4)_2$, and 2/8 $MgSO_4$. Transpiration and green and dry weights of tops were generally higher in the water cultures, but greatest root development occurred in the sand cultures. In general, transpiration records over the entire period of growth, and dry weight records of tops and roots, show parallel variations.—R. B. DUSTMAN.

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[6] SHELFORD, V. E., and GAIL, F. W., A study of light penetration into seawater made with the Kunz photo-electric cell, with particular reference to distribution of plants. Publ. Puget Sound Biol. Sta. **3**:141–176. 1922.

[7] BAKKE, A. L., and ERDMAN, L. W., A comparative study of sand and solution cultures of Marquis wheat. Amer. Jour. Bot. **10**:18–31. 1923.

Hydrogen ion injury.—Certain abnormalities and retardations of root development in nutrient solutions made up to contain relatively high amounts of dihydrogen phosphates have been traced by Miss ADDOMS[8] to hydrogen ion injury. She deserves credit for having employed the dark field illuminator in making the injuries visible. The injurious effects seem to be due to a coagulation of the protoplasm of the root hairs. The range studied runs from P_H 3.94 to 3.47, a rather narrow range, and it is possible that the experiments did not run long enough to show whether the least concentration of hydrogen ion used is really harmless to protoplasm. The statement that there can be no direct relation between the hydrogen ion concentration of nutrient solutions and the yield of plants grown in them may need interpretation. If it is meant that there is no correlation between hydrogen ion concentration and yield, one would want statistical data as proof. The definitions of gel, coagulation, and flocculation appended in a foot note seem to the reviewer not in accord with the common use of the terms. Some authors describe reversible gel formation in the protoplasm.—C. A. SHULL.

Availability of mineral plant food.—A modification of the generally accepted views of mineral availability is offered by COMBER.[9] In connection with the assumption that all nutrients must dissolve in soil water before absorption, certain inconsistencies are pointed out. These include the relation of the composition of the soil solution to the mineral elements absorbed and the water transpired by the plant, the absorption of iron, and the availability of phosphates. Feeding in the soil and in nutrient solutions is held to be unlike. The possibilities are suggested, first that the plant may absorb colloidal particles, and second, that there may be a union of the root hair with the soil particles, through their respective hydrophilous colloids, in such a way that the two form one system through which the dissolution of the soil particle by the organic matter of the root hair is more readily accomplished.—R. B. DUSTMAN.

Wild flowers of South Africa.—An attractive magazine[10] on much the same lines as the well known CURTIS' *Botanical Magazine* is now in its third year. It contains hand colored plates of the flowering plants indigenous to South Africa, with accompanying botanical descriptions. The illustrations are mostly from drawings by Miss K. A. LANSDELL and Miss S. GLOVER, while the descriptions have been prepared by E. PERCY PHILLIPS, botanist in charge of the National Herbarium at Pretoria. The current volume begins with plate 81, illustrating *Clematopsis Stanleyi*, a small shrub of the Ranunculaceae. The quality of the illustrations seems to compare favorably with that of the publication after which it was modeled, while the remarkable diversity of the flora of South Africa affords many unique flowers to attract the attention of artists as well as botanists.—GEO. D. FULLER.

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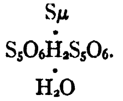
[8] ADDOMS, RUTH M., The effect of the hydrogen ion on the protoplasm of the root hairs of wheat. Amer. Jour. Bot. 10:211–220. 1923.

[9] COMBER, N. M., The availability of mineral plant food. Jour. Agric. Sci. 12:363–369. 1922.

[10] The flowering plants of South Africa, edited by T. B. POLE EVANS. Quarterly. Pub. by Specialty Press of South Africa, Johannesburg and Cape Town. 60*s* annually.

Defoliation and blossom bud formation.—ROBERTS[11] finds that defoliation has a marked inhibiting effect on the formation of flower buds. There was a close relation between the removal of fractional parts of the leaf and the number of buds formed, the more of the leaf removed the greater the inhibition. While the effect is largely localized at the node where defoliation takes place, there is some effect in reducing the number of buds formed at adjoining nodes. The length of the spurs growing at nodes defoliated the preceding season was less than in the case of spurs growing at undefoliated nodes. It is suggested that this may be due to the greater nitrogen reserve of the undefoliated nodes. It would be interesting to study further the chemistry of the defoliated and undefoliated nodal tissue. This will no doubt be done in later work. The present report is part of the preliminary work that has been done on the general problem of the formation of buds by fruit trees.—S. V. EATON.

Fungicidal action of sulphur.—Sulphur has been much used as a spray material in the control of plant diseases without our knowing in what way it exerted its toxic effects. YOUNG[12] has made a careful study of its action, and finds that the toxic compound which makes it a good fungicide is pentathionic acid, $H_2S_5O_6$. The pentathionic acid forms an excellent adsorption medium to bind together $S\mu$ and water into a hydrophilic colloidal sulphur, as represented by the formula  $\begin{array}{c} S\mu \\ \bullet \\ S_5O_6H_2S_5O_6 \\ \bullet \\ H_2O \end{array}$. This hydrophilic colloidal sulphur exhibits toxicity only at P_H 4.2–5.4, because the pentathionic acid is unstable at hydrogen ion concentrations on either side of this range. This work explains the effectiveness of various colloidal sulphur sprays which have been introduced in the last few years in Australia and Europe.—C. A. SHULL.

Lichens and glass.—Miss MELLOR[13] has recently added to her interesting contributions to our knowledge of the ability of lichens to etch the surface of glass windows. Her previous work, noted in this journal,[14] is now supplemented by further evidence. Twenty-three species and varieties are recognized as frequenting this curious habitat, of which *Diploicia canescens* is most abundant.

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[11] ROBERTS, R. H., Effect of defoliation upon blossom bud formation. Wis. Agric. Exp. Sta. Res. Bull. 48. pp. 15. *figs. 5.* 1923.

[12] YOUNG, H. C., The toxic property of sulphur. Ann. Mo. Bot. Gard. 9:403–435. 1922.

[13] MELLOR, ETHEL, Lichens and their action on the glass and leadings of church windows. Nature 112:299–300. 1923.

[14] BOT. GAZ. 73:423. 1922.

Some of Miss MELLOR's conclusions have been called in question by HEATON,[15] who regards the decay of the glass of old stained glass as the cause of the presence of lichens, rather than the reverse. This criticism brings a reply from Miss MELLOR,[16] whose contention that lichens cause disintegration of the surface of stained glass and the production of pits seems to be well supported by her evidence.—GEO. D. FULLER.

Vegetation of Himalayas and of Corsica.—The first[17] of two recent numbers of the Vegetationsbilder contains excellent illustrations of monsoon forest, *Quercus incana* forest, *Pinus longifolia* forest, and of *Euphorbia Royleana* scrub, all from the slopes of the western Himalayas.

The second number[18] is devoted to the vegetation of Corsica, and gives illustrations of *Nerium oleander*, *Arundo donax*, *Gomphocarpus fruticosus*, and *Helleborus trifolius*, together with photographs of macchie, forests of *Pinus nigra*, *Abies alba*, and of *Fagus silvatica*.—GEO. D. FULLER.

Sexual state of plants.—SCHAFFNER,[19] in continuation of his investigation of sexuality, has published results of work with *Acnida tamariscina*, *Thalictrum dasycarpum*, *Acer saccharinum*, *A. rubrum*, *Thalictrum dioicum*, and *Aesculus glabra*. He is convinced that the various expressions of sexuality can be arranged in "a progressive series of many stages in respect to the intensity and fixity of the sexual state, all depending on a fundamentally similar physiological activity." He gives a sequence of eight of the more prominent stages, beginning with typical bisporangiate species, and through intermediate stages culminating in dioecious species with no intermediates and no sex reversal.—J. M. C.

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[15] HEATON, N., Lichens and their action on the glass and leadings of church windows. Nature, 505–506. 1923.

[16] MELLOR, ETHEL, Lichens and their action on the glass and leadings of church windows. Ibid. 506. 1923.

[17] KENOYER, L. A., Waldformationen des westlichen Himalaya. Vegetationsbilder, Karsten and Schenck 15: heft 1. *pls. 1–6.* 1923.

[18] RIKLI, M., and RUBEL, E., Korsika. Vegetationsbilder, Karsten and Schenck 15: heft 1. *pls. 7–12.* 1923.

[19] SCHAFFNER, J. H., Observations on the sexual state of various plants. Ohio Jour. Sci. **23**: 149–159. 1923.

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DETERMINATION OF SEX IN ELODEA

José K. Santos

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 317

(WITH PLATES XXIII–XXVII AND EIGHT FIGURES)

In studying the reduction mitoses in *Elodea gigantea* (38), some differentiation was found among its chromosomes, but only male plants were available; consequently, it seemed desirable to investigate our native species, *E. canadensis*, in which both male and female plants are abundant.

My previous paper described the work already done on the determination of sex in plants, beginning with STRASBURGER (44), who reviewed the theory that sex is determined by the surrounding conditions; the cytological work published by DARLING (10) on *Acer negundo*, who claimed that he found an unequal distribution of chromatin in the nuclei resulting from the homotypic division; the work of MOTTIER (33), who studied the same plant but obtained different results; and the experimental and cytological investigation of ALLEN (1) on *Sphaerocarpus*, with its direct relation to the experimental work of C. and R. DOUIN (15) on the same genus; those of the MARCHALS' (28, 29) on mosses; CORRENS (9) on *Bryonia dioica* and *B. alba;* NOLL (34) on *Cannabis;* and STRASBURGER (45) on *Mercurialis annua*, *Melandrium album*, and others.

Material and methods

The material used in this investigation was collected in the early part of June 1922, from Wolf Lake, about fourteen miles from Chicago, where STRASBURGER'S material was collected. Ten sets were fixed in the field on different days and at different times of the day; and four sets were pickled in the greenhouse, where a quantity of the plant was kept. In addition, some material collected in August 1921 was used.

The fixing reagent was 1 per cent chromo-acetic acid, with about ten drops of 1 per cent osmic acid to 50 cc. of the solution. The material was imbedded in 52° C. paraffin, and cut 3-4 μ thick, with some 10-15 μ thick, and stained in Haidenhain's iron-alum haematoxylin with methyl orange.

Heterotypic prophase to synizesis

The reduction divisions in *E. canadensis* agree in almost every respect with those of *E. gigantea*, as described in my former paper (38), but all the structures are smaller. The pollen mother cell following the last mitosis of the archesporial region is distinctly polygonal in outline in cross and longitudinal sections of the anther. Its cytoplasm is dense, and more or less uniformly granular. The nucleus may be in the center of the cell or very close to one side. Its structure consists of a chromatin network or mesh, with nodelike structures or beadlike enlargements in the intersections of the chromatin threads. Sometimes these fine chromatin threads and nodes are observed in pairs. According to Miss DIGBY (13), this is the first indication of the reassociation of the chromosome halves (threads) which separated during the preceding telophase. Fig. 1 illustrates the earliest definite prophase of the first reduction division. The nucleolus may be vacuolated or not, and may be in the center or near to the periphery of the nucleus.

As the nucleus enlarges the reticulum loses its reticular character, the chromatin nodes become larger and closer together, and the threads become thicker. This is perhaps due to the fact that the chromatin threads are gradually contracting and pairing. I was unable to find a place at this stage where the chromatin threads are not doubled. Whether or not this pairing is due to mere "chance parallelisms," as claimed by CLELAND (8), the results in *Elodea* seem to agree with the findings of Miss DIGBY (13) in *Osmunda*. Fig. 2 shows the gradual pairing of the threads, and in fig. 3 this pairing has become more evident. From this stage the nuclear contents are slowly withdrawn from the nuclear periphery, which indicates that synizesis is about to take place. In figs. 3 and 4 the appearance of a clear space on one or two sides of the nucleus is represented. This period of development of the mother cell is brought about by the contraction of the nuclear contents (chromatin threads) and by the enlargement of the nuclear cavity (38).

SYNIZESIS.- As the nucleus advances into synizesis, the pairing and contraction continue, until at last a more or less compact ball of chromatin at one side of the nucleus is formed. In the majority of cases, but not always, the synizesic knot lies on that side of the nucleus which is nearest the periphery of the cell. This is the most critical stage in the development of the mother cell, and it remains in this condition for a comparatively long period. In *Zamia* this stage lasts for several days, the structure of the knot being so tight that sometimes it looks like a granular precipitate from a chemical. reaction entangled in a mass of threads. In most cases one or two loops of threads project from the knot. A careful examination of thin tangential sections of the chromatin ball, however, shows that the chromatin granules were brought close together by the contraction of the threads. In many cases these chromatin nodes or granules run in rows side by side. Fig. 5 represents a typical knot in which some of the chromatin threads are projecting in loops from the mass. As this process advances, the spireme becomes more and more distinct and uniform. Miss DIGBY believes that during this period of the life of the mother cell, the association of threads in pairs, prepared during the presynaptic stages, is consummated. All the evidences in *Elodea* seem to support this theory. The spireme threads during the later part of synizesis show a double character all the way through, and become very much thicker than before. Up to this stage no material change is observed in the shape and size of the nucleolus, except that its vacuoles are more pronounced, and occasionally protuberances or budlike structures are noticed coming out from its surface.

OPEN SPIREME.—Soon after the maximum synizesic contraction is attained, the knot loosens its structure gradually, and the closely doubled threads begin to extend slowly throughout the nuclear cavity, until they are more or less uniformly distributed (figs. 6–8). Meanwhile the spireme becomes thicker, and appears as a homogeneous, smooth, single, and continuous filament, although in some places free ends may be observed. A critical examination of the spireme at this stage, however, reveals the fact that in many cases the chromatin nodes are found in two close and compact rows, which appear single at first glance.

SECOND CONTRACTION.—Immediately after the distribution of the spireme in the nuclear cavity, loops are formed (fig. 9). These loops are especially characteristic before the partial segmentation and second contraction take place (figs. 10, 11). Shortly after the numerous loops are thrown and spread through the nuclear vacuole, the spireme passes into the condition known as second contraction, and during this period the chromatin filament becomes thicker and thicker, until its double nature can no longer be detected. I believe that this is the most critical part of the nuclear development. The loops become rearranged, their sides begin to approach one another, and they gradually become detached. The segments of the spireme become entangled, and it is almost impossible to determine the nature and number of the individual pieces. Fig. 12 shows a stage preparatory to second contraction, in which the two ends of some of the loops have twisted around each other. Figs. 13 and 14 represent the character of the second contraction in *Elodea*. Occasionally at this stage a constriction is observed at the distal end of the loop, which indicates that each side of the loop represents a univalent chromosome. During the later part of the second contraction, and before the individual segments are thrown to the periphery, the pollen mother cells, which during the resting period and up to the later stage of synizesis were packed closely, are beginning to round off and separate from one another.

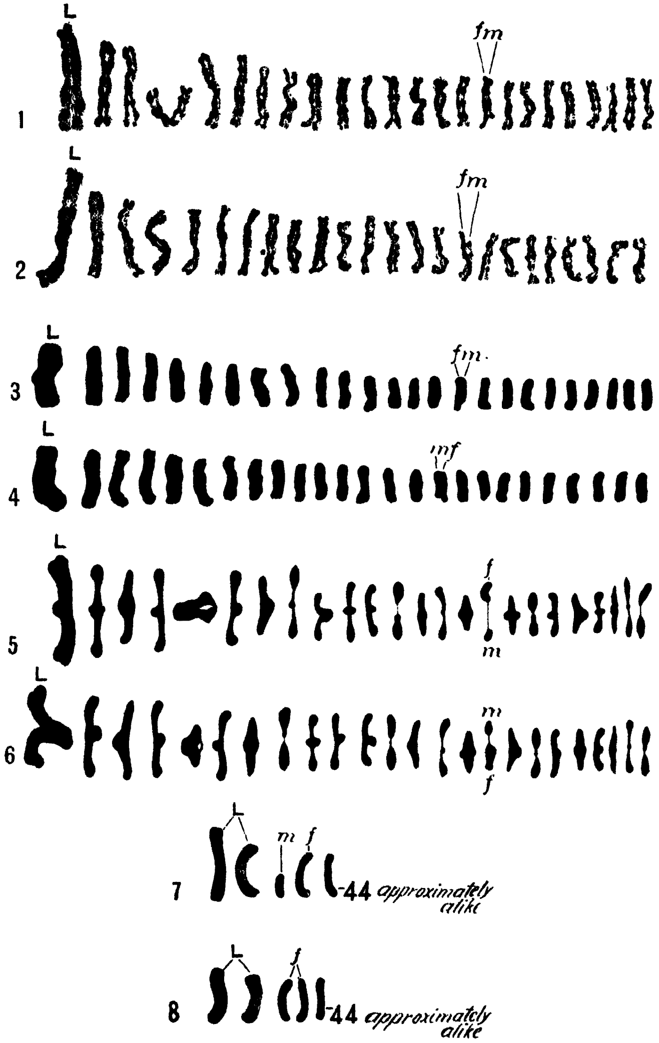
Diakinesis

The knot formed by the second contraction does not seem to be of long duration, judging from the scarcity of this stage, but soon loosens, and the young bivalent chromosomes are thrown toward the periphery of the nucleus. This is indicated in fig. 15, in which the young heterotypic chromosomes are just coming out from the second contraction. Some of them are in the form of 8's, due to the twisting about each other of the parallel sides of the loops; some still have the *U* shape; and others are composed of two straight or slightly curved rods lying side by side and sometimes slightly twisted about each other; or the two segments adhere at one end, taking a more or less *V* shape. Closely following the stage shown in fig. 15 is that of a complete distribution of the partially condensed bivalent chromosomes shown in fig. 16, in which some of the chromosomes are clearly marked out, while others are still in the process of organization. Beginning at this stage, a critical study was made of the differentiation among chromosomes. The numerous counts made agreed remarkably with the number of bivalent chromosomes in *Elodea gigantea;* and even the characteristic unequal pair and the large or giant chromosome described in my earlier paper (38) were found. In text figs. 1 and 2 are two groups of the young heterotypic chromosomes, in which it is easy to distinguish the differences in size. Fig. 17 represents a later stage of diakinesis. This is one of the most favorable stages for the observation of the unequal pair of chromosomes, when one is fortunate enough to get the proper orientation, for sometimes the chromosomes are close to the periphery and point upward, both of which conditions make it difficult to recognize the exact outline of the two members of the pair.

It is important to notice that during the later period of the second contraction, up to the later period of diakinesis, the nucleus becomes more and more vacuolated and seems to be thin and empty. The cytoplasm near the nuclear membrane in the meantime becomes denser, making the nuclear wall seem thick and irregular in outline. This is the first indication that the spindle fibers are about to appear. At the time the spindle fibers appear, the nuclear membrane and the nucleolus disappear simultaneously. This has led to the suggestion that both the membrane and the nucleolus have been used in the formation of the spindles (38), or that in some way they contribute to the formation of spindle fibers, as has been emphasized by HARPER (18), GATES (16), DAVIS (12), and Miss DIGBY (13). On the other hand, LAWSON (26) believes that the nuclear membrane does not break down and is not used up in spindle formation, but that it becomes applied to, and completely envelops the surface of each chromosome. I have come to the conclusion, however, that not all the material for the spindle fibers is stored in the nucleolus and nuclear membrane, nor do these structures disappear completely; but their material is deposited in the cytoplasm, particularly on the sides of the nucleus toward the two poles, during the later period of the telophase. They reappear at the time the nuclear membrane is broken down, or even before the disorganization of the nuclear wall. Their structure in the early period of their formation shows that they consist of rather coarse threads with uniform nodal enlargements which look like a string of beads. When close together they give the general appearance of the structure of the cytoplasm. The beads disappear, however, as soon as the spindle fibers are stretched toward the two poles. Whether this beadlike structure is due to nucleolar exudation or disintegration I have not been able to determine. Fig. 18 is a longitudinal section of the nucleus, showing the young spindle fibers emerging from at least four parts of the nucleus. Fig. 19 is similar, except that it is cut crosswise. At this stage the bivalent chromosomes are greatly condensed and well distributed throughout the nuclear vacuole. The fission between the two monovalent chromosomes that composes the bivalent chromosome is practically indistinguishable. Owing to the irregular position of the chromosomes, it is very hard to detect the uneven pair, but the giant one is evident.

Heterotypic mitosis

While the spindle fibers are developing and the nucleolus and nuclear membrane are disappearing, the heterotypic chromosomes undergo a process of condensation, until they are reduced to about one-half or less of their original size shown in the late diakinesis stage. This stage is represented in fig. 20, in which the fibers are stretching themselves and have lost their beaded character, while the chromosomes are moving toward the equatorial plate. Detailed drawings of the chromosomes of this stage are shown in the text figs. 3 and 4. An oblique polar view of the chromosomes arranged in the equatorial plate is represented in fig. 21. They exhibit the same differences in size and shape and number as was observed



FIGS. 1–8.—Eight chromosome groups of *Elodea canadensis:* figs. 1 and 2 taken from early stage of diakinesis, with 24 bivalent chromosomes each; figs. 3 and 4 from late diakinesis stage or early metaphase; figs. 5 and 6 from metaphase stage, also with 24 bivalent chromosomes; fig. 7 from stem tip of male sporophyte, with 48 univalent chromosomes; and fig. 8 from stem tip of female sporophyte, with same number of chromosomes as male.

in *Elodea gigantea*. The large chromosome is evident as well as the unequal pair.

Soon after the 24 bivalent chromosomes become arranged in a definite plate, at which time they appear more or less globular and oblong ovoid, the fibers begin to pull apart gradually the two halves of each bivalent chromosome. A typical side view of the metaphase of the heterotypic mitosis is represented in fig. 22, while fig. 23 shows late metaphase or early anaphase. In these two stages the giant chromosome is obviously splitting into two equal halves; occasionally it lags somewhat. On the other hand, the chromosome with unequal members is characterized by dividing earlier than the others. Frequently the small member is observed moving ahead, so that it is distinctly in advance of the rest of the chromosomes of its group. The point of attachment of the spindle fibers does not seem to be definite. Generally it is at the end, but sometimes it is a little toward the middle (text figs. 5, 6). The dual nature of the daughter chromosomes is practically unrecognizable, but in some instances may be traced more or less at their tips.

Two stages of the anaphase, one following the other, are seen in figs. 24 and 25, in which most of the daughter chromosomes (somatic chromosomes) are *V*-shaped, with the apex of the *V* attached to the spindle. In both stages the giant chromosomes and the small member of the uneven pair are easily seen. The double nature of the haploid chromosomes is more or less distinguishable at their tips. These pass entire to the pole, where they form a little cluster in the anastomosing condition (fig. 26). Then the nuclear membrane, followed later by the nucleolus, begins to appear, and the wall between the daughter cells is gradually formed by the thickening of the middle part of the spindles. The daughter nuclei enlarge immediately and assume an ovoid form. They are characterized by the fact that they do not reach a true resting condition, but soon prepare for the second division.

Homotypic mitosis

The life of the nuclear membrane of the daughter nuclei, resulting from the heterotypic mitosis, is of short duration, because the second meiotic division follows quickly. As the two nuclear membranes break down and the two nucleoli disappear, the two homotypic spindles appear simultaneously. These may lie parallel or at right angles to each other, or sometimes in a *V*-shaped position (figs. 29, 30). Fig. 28 shows two groups of chromosomes in the polar view of the two daughter nuclei arranged in the equatorial plate. Even in this stage the large chromosome and the small one from the unequal pair are noticeable. In this second division all the daughter chromosomes are split equally, and, as usual, the small chromosome divides earlier than the others (figs. 29, 30). Fig. 31 is at about the same stage as fig. 30, and illustrates the other arrangement of the two homotypic spindles. The four granddaughter nuclei are formed about as the daughter nuclei in the first mitosis. Two of them, however, are slightly smaller than the other two (fig. 32). The nuclei grow rapidly and pass quickly into resting stage. At this stage the difference in size of the nuclei is almost imperceptible. Fig. 33 is an older stage in which the four nuclei are almost alike. To learn whether the mature pollen grains are affected by such differences as were observed in the size of the four young granddaughter nuclei, I made the following measurements of the microspores collected at the time they were shed:

FIRST PAIR	SECOND PAIR
Average diameters	Average diameters
102.73 μ×97.35 μ	94.11 μ×85.65 μ
101.88 μ×97.36 μ	93.77 μ×85.67 μ

Each of these averages is calculated from twenty-five measurements. It is evident that two of the resulting microspores are slightly larger than the other two. Although this difference is rather small, it is very significant in its relation to the unequal distribution of the chromatin, due to the small chromosome from the uneven pair in the heterotypic mitosis.

It is interesting to notice that the four microspores at an early stage are within the wall of the mother cell, but soon the wall disappears, so that they are no longer inclosed. At this stage the young tetrads enter upon a period of rapid enlargement. One peculiarity of the tetrad is that its four members do not fall apart, but remain attached and are ultimately shed from the sporangium still firmly joined together. They have a strongly cutinized exine and a well developed entine.

Somatic mitosis of formation of tube and generative nuclei

TELOPHASE TO RESTING STAGE.—Soon after the chromosomes in the homotypic mitosis have reached the poles and have formed a more or less compact knot, the nuclear membrane begins to appear. At this stage the chromosomes seem to lose temporarily their individuality, but as soon as the rapid growth of the nucleus proceeds, their individual boundaries again become visible. They appear much elongated, lying parallel to each other across the nuclear cavity, sometimes with much regularity, with their sides cohering at various points. While the enlargement of the nucleus is in progress the nucleoli appear suddenly, and the chromosome bands become vacuolated (figs. 34, 35). GRÉGOIRE (17), in his work on *Allium*, has shown that each chromosome shows two parallel lines, composed of threads or granules. In this somatic mitosis of *Elodea* it seems that the same process is taking place. The splitting of each chromosome into two halves is evident. This is in accord also with the idea proposed by Miss DIGBY and others. The vacuolization and splitting continue until a beaded chromatin framework or mesh is formed, which is the stage commonly known as "resting." This condition in the pollen nucleus seems to persist for a comparatively short period, and soon the nuclear contents begin to reorganize by the pairing of the chromatin threads (fig. 36).

CHROMOSOME FORMATION.—The pairing of the chromatin threads in the pollen nucleus of *Elodea* seems to occur with much regularity. I have examined a great number of them at this stage, and was unable to find a single one in which the young spireme was not doubled. The chromatin granules appear also with regularity in two close parallel rows (figs. 38, 39). As the nucleus enlarges, the spireme becomes more distinct and thicker and apparently loses its double nature. Once in a while one can find, here and there, some free ends. This perhaps is due in some places to the sectioning. The granules also appear single, but double the size of those in the resting stage (fig. 40). At this stage the spireme seems to be almost uniform in diameter and almost straight, radiating in different directions across the nuclear cavity. The first sign to be noticed before segmentation of the spireme is that it becomes very irregular in diameter, that is, in certain places it is very thick and wavy, but thin in the other places (fig. 41). Closely following this stage is that shown in fig. 42, in which the individual segments are distinctly seen separating from each other. Fig. 43 shows the partial condensation of the chromosomes which have become individualized.

METAPHASE TO TELOPHASE.—When the maximum condensation has been attained by the chromosomes, and when the nuclear membrane and the nucleolus have disappeared, the chromosomes arrange themselves in the usual manner in the equatorial plate. Figs. 44 and 45 are two polar views taken from the same tetrad, in which it is easy to count 24 chromosomes in each group. This is the best stage in the formation of the pollen grain for counting the number of chromosomes, for they are not so crowded together. The difference in size of the chromosomes is easily seen. In fig. 44 the large chromosome (*L*) and the small one (*m*) are evident; while in fig. 45 only the large one is present. It is easy to conclude that they are not pairmates.

Typical side views of the metaphase stage are represented in figs. 46–49. It was fortunate, after examining many tetrads, to get these four nuclei in division and with proper orientation, so that I was able to trace and identify the large (*L*) and small (*m*) chromosomes. Judging from the presence of these two types of chromosomes, it is evident that figs. 46 and 47 are from the one nucleus, and figs. 48 and 49 from the other. From the metaphase the chromosomes pass to their respective poles in the usual way. Late anaphase is shown in figs. 50 and 51. Both nuclei are from the same tetrad. In fig. 50 the giant (*L*) chromosome is indicated, while in fig. 51 both the large (*L*) and small (*m*) one can be identified. Closely following this figure is the stage shown in fig. 52, with the appearance of the nuclear membrane, the resolution of the chromosomes in the usual fashion, and the beginning of the formation of the temporary wall between the generative cell and tube cell. A more advanced stage is shown in fig. 53, in which the thick wall (*w*) of the pollen, the generative cell (*g*), and the tube nucleus (*tn*) are passing into the resting stage. The spindle fibers remain until this stage but soon resolve themselves into reticulate cytoplasm.

Chromosomes of male and female sporophytes

A careful examination of the chromosomes of the male and female sporophytes showed that there are 48 somatic chromosomes in each of their dividing cells in the meristematic tissue. On account of the great number of chromosomes it was difficult to count them, and to get a proper orientation for the examination of their differences in size. After examining a number of the root and branch tips, however, I found that in most cases there were 48 somatic chromosomes in both male and female. In some instances the numbers 46 and 47 were met, but I accounted for this variation by their frequent overlapping. Moreover, since 48 is double the number of the bivalent chromosomes in the meiotic mitosis, I believe it is the exact number for the somatic chromosomes.

In examining critically the size of the chromosomes of the male, among the 48 of them, two are extraordinarily large, exceeding in length and in width the other chromosomes, while one of them is very small. The latter is not always easy to find except where favorable orientation and good scattering of the chromosomes are obtained. Evidently, in the reduction division the two large chromosomes unite with each other, and the small one unites with a chromosome of about the same size as the rest, and thus the unequal pair is formed. Fig. 54 represents a typical group of somatic chromosomes from a branch tip of the male sporophyte. On the other hand, in the female sporophyte, in most of the cases, out of the 48 chromosomes two of them are as large as those found in the male sporophyte, and the rest have approximately the same size (figs. 55, 56). Fig. 55 is taken from a section of a very young leaf near the tip of the branch, in which each of the large chromosomes has divided into two halves; and fig. 56 is also taken from a branch tip, showing the two large chromosomes corresponding to those found in the male sporophyte.

Relation of chromosomes to sex

Elodea canadensis shows an extremely close agreement in the general history of the chromosome group, and especially in the behavior of the unequal pair and the giant chromosome with the condition found in *E. gigantea*. In the prophase of the somatic mitosis of the male and female sporophytes, I found in both cases 48 univalent chromosomes. In the male, out of the 48, two are extraordinarily large, exceeding the other chromosomes both in length and width, and one is very small (fig. 54). In the female plant there are two large chromosomes, corresponding in size with those in the male, and 46 chromosomes approximately alike (figs. 55, 56). On the other hand, in the maturation division of the male, I found 24 bivalent chromosomes, which is evidently half of the number in the somatic. Out of these 24, one exceeds the other chromosomes in length and width, and another is characteristic because of the unequal length of its component elements. It is clear that the two large chromosomes of the somatic nuclei have united, and the small one has paired with a chromosome of about the same size as the chromosomes, to which the term autosome is applied, resulting in an uneven pair. These separate from each other in the first division, in which one of the daughter nuclei receives one giant chromosome, the larger member of the unequal pair, and 22 autosomes; while the other daughter nucleus receives one giant chromosome and a very small one (from the unequal pair), and 22 autosomes. In the second maturation division each chromosome of the daughter nuclei divides equally, and thus two of the four resulting daughter nuclei receive one giant and one small chromosome and 22 autosomes; while the other two receive one giant chromosome, the larger member of the unequal pair, and 22 autosomes. It is evident, therefore, that there is an unequal distribution of chromatin as in the other species of *Elodea*. This unequal distribution of the chromatin affects in some way the size of the resulting four nuclei during their early stages. Two of them are slightly smaller than the other two, and by re-examining the slides of *E. gigantea* I found the same differences, and they do not, as stated in my previous paper (38), look alike. The resulting pollen grains show also slight differences in size, two of them being smaller than the other two, as previously demonstrated.

The presence of the unequal pair in the meiotic division, and the small chromosome in somatic mitosis in the male sporophyte, show that the unequal pair has a direct bearing upon the determination of sex. The behavior of the two members of the unequal pair agrees remarkably with that of the *Y* and *X* chromosomes in *Drosophila* (STEVENS **43**, MORGAN **32**, METZ **30**, and others); the heterochromosomes or idiochromosomes in *Lygaeus* (WILSON **48, 49**); *Euschistus* (MONTGOMERY **31**); and other insects. It also agrees with the *X* and *Y* chromosomes found in *Sphaerocarpus*, a dioecious bryophyte, by ALLEN (**1**). In view of this similarity, undoubtedly the chromosomes in the unequal pair bear sex characters, as in the insects and *Sphaerocarpus*. For this reason the small chromosome is marked *m*, indicating its male character, and its mate *f*, indicating its female character. The large, or giant, chromosome is marked *L*. This giant chromosome may have some special function in heredity, but so far as my observations are concerned, it behaves like an ordinary autosome.

Discussion

In regard to the question of sex determination in *Elodea* and its relation to the accumulated evidences found in animals as well as in plants, I have very little to add to what was given in my previous paper. The unequal pair evidently plays a very important rôle in the distribution of the chromatin to the four microspores. This unequal distribution of chromatin to the microspores is doubtless responsible for the 1 : 1 ratio of the sex distribution in plants, as has been observed in animals. WILSON (**48, 49**), however, believes these quantitative differences cannot always be considered as primary sex determining factors, for he found that such a view is inapplicable to cases like *Nezara*, *Oncopeltus*, or *Metapodius*. In most of the cases studied in animals, however, the unequal distribution of the chromatin prevails. So, in the case of *Elodea*, I firmly believe that the *m* and *f* chromosomes function in the determination of sex.

In my previous paper I have quoted the five classes of the much studied cases in animals published by WILSON (**48, 49**), in his comparative review of the types of sexual differences of the chromosome groups. In his classification, despite the apparent diversity of the types, all are in accord with the principle that the spermatozoa are of two kinds, equal in number, respectively male producing and female producing. The case of *Elodea*, as already indicated, agrees remarkably with those in the second group, and at the same time it is in accord with the principle.

The question of experimental evidences in sex determination in plants has been discussed in my previous paper, which emphasized the work of the MARCHALS (**28, 29**) on mosses; DOUIN (**14**) and ALLEN (**1**) on *Sphaerocarpus;* CORRENS (**9**) on *Bryonia dioica* and *B. alba;* NOLL (**34**) on *Cannabis;* STRASBURGER (**45**) on *Mercurialis annua* and *Melandrium album;* and others. In all these investigations it was found that the sex ratio is about 1:1, or half male and half female. By changing the external conditions, they claim that they were not able to change that ratio.

The monoecious plants are quite abundant in the plant world, but the dioecious ones are also many. The important question is whether the dioecious plants have been derived from the monoecious ones. Lately some strong evidences that the dioecious plants have been derived from the monoecious plants have been published. STRASBURGER (**45**), in a general discussion of his investigations on *Elodea, Mercurialis,* and other plants, has summarized the situation in plants, the report being well translated by SHARP (**42**). In my previous paper I have given ALLEN'S and SHARP'S views in regard to the separation of sex factors in dioecious seed plants; and also COLLIN'S (**6, 7**) results in his experiments on *Funaria hygrometrica,* a dioecious moss, from which he was able to produce a monoecious plant.

Important results have come to light in the last few years, bearing upon the problem of experimental alteration of the sex ratio. Although we know that in most animals the sex ratio runs approximately 1:1, occasionally some deviations have been observed, which have led to some experimentation. Among the important results obtained in animals are those of HERTWIG (**19, 20**) and KUSCHAKEWISCH (**25**). Under normal condition the eggs of a frog give rise to male and female individuals in equal numbers; but by allowing the eggs, before fertilization, to take up an abnormal amount of water, the number of males was considerably increased, sometimes even to 100 per cent. On the other hand, Miss KING (**21, 22, 23, 24**), by lowering the water content of toad eggs, was able to increase the proportion of females. Similar results have been obtained with *Dinophilus* (MALSEN **27**) and *Hydatina senta* (WHITNEY **46, 47**).

In plants a number of experiments of similar nature have given more or less similar results. According to SHARP (42), however, these are less conclusive than those in animals, for the reason that most of the experiments have been carried out with angiosperms, in which intersexes are common. Among the plants that have been worked out, and that showed intersexes, may be cited the following: *Cannabis sativa* (PRITCHARD 36); *Myrica Gale* (DAVEY and GIBSON 11); *Arisaema dracontium* (SCHAFFNER 41); *Cannabis sativa*, *Salix amygdaloides*, and *Morus alba* (SCHAFFNER 39, 40); *Plantago lanceolata* (BARTLETT 3; CORRENS 9); *Mercurialis annua* (YAMPOLSKY 51, 52). So far as *Elodea* is concerned, only one case, reported by HITCHCOCK, showed stamens and carpel in the same flower.

In view of the evidences obtained by the morphologists and physiologists, two theories have been proposed, both with strong supporters. One theory claims that sex is determined and controlled by the presence of the accessory or heterochromosomes; while the other claims that sex is determined and controlled by the environmental factors. It is interesting to notice, however, that those who belong to the morphological side confine their studies practically to that side; and that those who take the physiological view, pay little or no attention to internal structure. I agree with the statement of Professor SHARP (42) in his discussion of sex, that "It is beyond question that the two manifestations of sexual differentiation, the physiological and the morphological, are both of importance and cannot entirely be irreconcilable: our task is to determine their relative significance and to discover the nature and degree of their mutual independence." Any experimental studies should be accompanied with a critical cytological investigation. The effect of the environmental condition should not be looked upon from the external character of the individual only, but also in its effect upon the structure of the cell. One apparently may get some changes in form, but in reality the changes may be produced by changes in the chromatin.

Summary

1. The resting nucleus of *Elodea canadensis* consists of a chromatin network or mesh with chromatin beadlike structures in the intersections of the chromatin threads.

2. Before synizesis there is a parallelism of the threads and beads. As the nucleus is in progress of enlargement, the reticulum loses its reticular character, and the pairing of the threads becomes more distinct.

3. The first indication that synizesis is about to take place is the appearance of a clear space on one or two sides of the nucleus, the thickening of the chromatin threads, and the enlargement of the chromatin nodes.

4. The phenomenon known as synizesis consists in the rapid growth of the nucleus and especially in the contraction of the nuclear contents into a more or less compact mass, which may or may not include the nucleolus. This stage lasts for a long period.

5. After synizesis the knot loosens its structure, and the closely doubled threads (spireme) begin to extend gradually throughout the nuclear cavity until it is more or less uniformly distributed.

6. After the distribution of the spireme in the nuclear cavity, loops are formed, after which the spireme passes into the second contraction, and then gradually becomes segmented.

7. The two ends of each of the loops, as they emerge from the second contraction, approach each other and sometimes twist about each other. These consist of two univalent chromosomes, arranged end to end. The univalent chromosomes in *Elodea*, therefore, are arranged telosynaptically.

8. As the 24 bivalent chromosomes emerge from the second contraction, they undergo a considerable condensation, until they acquire a more or less definite form. At this stage occasionally the *m* and *f* chromosomes are evident.

9. The nucleolus and the nuclear membrane disappear simultaneously, while the bivalent chromosomes are drawn to the equatorial plate and the spindles appear almost at the same time. Each of the bivalent chromosomes is attached to the spindle by one end, and the two constituent elements move apart. The giant chromosome splits lengthwise and forms two large daughter chromo-

somes; while the *m* and *f* constituents of the unequal pair separate from each other. The *m* chromosome goes to one pole, while the *f* chromosome passes to the other.

10. As the daughter chromosomes move toward the poles, they become *V*-shaped, with the apex attached to the spindle. The longitudinal split of the daughter chromosomes preparatory to the homotypic mitosis appears as they come very close to the poles.

11. The daughter nuclei resulting from the heterotypic division do not pass into a true resting stage, not reaching a true reticulum, but soon reorganize for the homotypic mitosis. As the nuclear membranes and the nucleolus disappear, the two homotypic spindles appear simultaneously. The univalent chromosomes arrange themselves in the equatorial plates and split equally lengthwise.

12. In the second division all the daughter chromosomes divide equally, so that two of the four resulting grand-daughter nuclei have received an equal share of the *m* chromosome, while the other two have received an equal share of the *f* chromosome from the unequal pair. The two that received the halves of the *m* chromosome have less chromatin than the two that received the halves of the *f* chromosome. Moreover, the two of the four resulting microspores that received the smaller amount of chromatin are slightly smaller than the two that received the larger amount.

13. The dual character of the univalent chromosomes becomes distinct from late anaphase to telophase. The fission between the two halves of the chromosomes becomes more evident as the nucleus proceeds to the resting stage.

14. Before the resting stage, a row of vacuoles appears along the fission of the chromosomes and splits them into halves or threads. As the nucleus enlarges, these are stretched and uniformly distributed in the nuclear cavity, in the form of a network or mesh, which becomes beaded.

15. At the resting stage the nuclear contents consist of a fine network of chromatin threads with chromatin enlargements in the intersections of the threads.

16. Soon after a short period of rest, the reticular character of the nucleus becomes disarranged. The chromatin threads with the chromatin nodes are brought together in parallel pairs, until

the spireme is well organized. This becomes thicker and irregular in diameter, and finally is segmented into 24 univalent chromosomes.

17. At the metaphase stage the giant *L* chromosomes, as well as the *m* chromosome, are easily seen. Each of the univalent chromosomes splits equally lengthwise, and the halves of the chromosomes proceed to their respective poles in the usual manner.

18. The somatic chromosomes of the male sporophyte are 48 in number. Out of the 48 univalent chromosomes, two are extraordinarily large, exceeding the other chromosomes in length and width; and one is very small.

19. The female sporophyte contained the same number of chromosomes as the male sporophyte. There are also two large ones that correspond in size to the two large chromosomes in the male, but the rest are approximately alike.

I wish to express my acknowledgment and gratitude to Professor CHARLES J. CHAMBERLAIN for proposing the problem, and also for suggestions during the course of the investigation.

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EXPLANATION OF PLATES XXIII–XXVII

All figures drawn with aid of camera lucida, using a Zeiss 2 mm. N.A. 1.4 oil immersion objective and 18X eye-piece.

PLATE XXIII

Fig. 1.—Typical resting stage of nucleus of pollen mother cell.

Fig. 2.—Older stage, showing chromatin threads gradually approaching each other side by side.

Fig. 3.—Beginning of prophase, in which in upper and lower part can be seen a clear space between nuclear membrane and reticulum.

Fig. 4.—Median section of nucleus, indicating gradual withdrawal of reticulum from nuclear wall, by contraction.

Fig. 5.—More or less complete synizesis, in which nucleolus has not been included in balled-up chromatin network.

Fig. 6.—Early stage of spireme, just emerging from synizesic ball.

Fig. 7.—Later stage in loosening up of spireme, with thread much thicker and well defined, appearing as a homogeneous, single, and continuous filament.

Fig. 8.—Complete hollow spireme with some loops beginning to form.

Fig. 9.—More or less tangential section, indicating characteristic loops preparatory to second contraction.

Figs. 10, 11.—Partial segmentation before second contraction.

Fig. 12.—Beginning of second contraction, in which ends of loops are twisted about each other.

PLATE XXIV

Figs. 13, 14.—Two complete second contraction stages.

Fig. 15.—Bivalent chromosomes emerging from second contraction; shape of nucleus becoming ovoid or oblong.

Figs. 16, 17.—Two successive diakinesis stages, showing about 24 bivalent chromosomes; *L*, large chromosome, and *mf*, unequal pair.

Fig. 18.—Median section, showing multipolar spindle emerging from nuclear wall and radiating across nuclear cavity; spindle at this stage beaded.

Fig. 19.—Another stage similar to that in fig. 18, except that it represents a cross-section of nucleus.

Fig. 20.—Spindle becoming bipolar and losing its beaded character, while bivalent chromosomes are moving toward equatorial plate.

Fig. 21.—Oblique polar view, in which chromosomes are arranged in equatorial plate; *L*, giant chromosome, and *mf*, unequal pair.

Fig. 22.—Complete side view of rather late metaphase, showing early separation of *m* and *f* chromosomes.

FIG. 23.—Early stage of anaphase or late metaphase, in which some of chromosomes are lagging, while *m* chromosome is distinctly ahead.

PLATE XXV

FIG. 24.—Typical side view of anaphase, showing most of daughter chromosomes in *V*-shape, with apex of *V* attached to spindle.

FIG. 25.—More advanced heterotypic anaphase: *m* chromosome not easily distinguished at this stage.

FIG. 26.—Early telophase, showing univalent chromosome forming cluster, while nuclear membrane begins to appear.

FIG. 27.—Late telophase, indicating very irregular shape of daughter chromosomes apparently going to resolve, but remaining more or less in this stage for a short period and not passing to true resting; wall between two daughter nuclei becoming distinct by thickening of middle part of spindle.

FIG. 28.—Polar view of daughter chromosomes preparing for second division; even at this stage *L* and *m* chromosomes distinguishable.

FIG. 29.—Side view of homotypic mitosis in metaphase stage; *M* chromosome obviously dividing ahead of rest.

FIGS. 30, 31.—Two different views of homotypic anaphases; in fig. 30, peculiarity of *m* chromosome displayed again.

FIG. 32.—Four young grand-daughter nuclei derived from single microspore mother cell lying in one plane; two decidedly smaller than other two; grand-daughter chromosomes resolving into resting condition.

PLATE XXVI

FIG. 33.—More advanced stage of grand-daughter nuclei, in which all are in prophase; difference in size almost imperceptible.

FIGS. 34, 35.—Two stages of grand-daughter nuclei, showing vacuolization and splitting of chromosomes proceeding to resting stage.

FIG. 36.—Typical median section of resting stage.

FIGS. 37, 38.—Two close stages, indicating parallelism of somatic chromatin threads.

FIG. 39.—More advanced, showing young spireme; double nature noticeable here and there.

FIG. 40.—Typical spireme, appearing single and uniform and coarsely beaded.

FIG. 41.—Spireme contracting and thickening in preparation for segmentation; notice irregularity of diameter.

FIG. 42.—Spireme segmenting into 24 univalent chromosomes.

FIG. 43.—Individual segment shortening and thickening.

PLATE XXVII

FIGS. 44, 45.—Two typical polar views of univalent chromosomes in prophase, taken from same tetrad; one shows *L* and *m* chromosomes distinctly, while other shows only *L* chromosome.

FIGS. 46–49.—Four nuclei in metaphase from same tetrad, in which two of them (48 and 49) show both *L* and *m* chromosomes, while other two show only *L* chromosome.

FIGS. 50, 51.—Two typical side views of anaphase, taken from same tetrad; one shows both *L* and *m* chromosomes, while other shows only *L* chromosome.

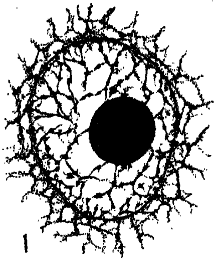
FIG. 52.—Tube nucleus and generative cell in telophase.

FIG. 53.—Tube nucleus proceeding to resting stage, generative cell about same stage, spindle resolving into cytoplasm, and thick wall of microspore.

FIG. 54.—Polar view of prophase stage from stem tip of male sporophyte, in which two *L* and *m* chromosomes well indicated.

FIG. 55.—Side view of anaphase stage from very young leaf in branch tip of female sporophyte; two *L* chromosomes noticeable.

FIG. 56.—Typical chromosome group in prophase, taken from branch tip of female sporophyte, in which two *L* chromosomes are shown.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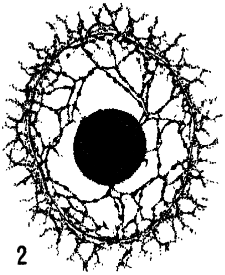
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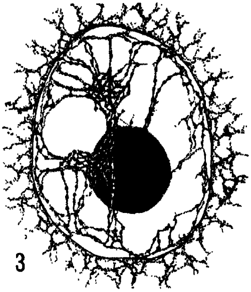
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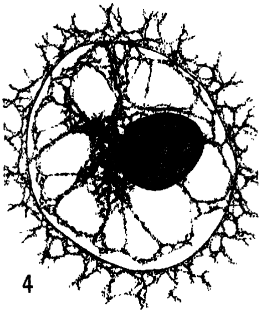
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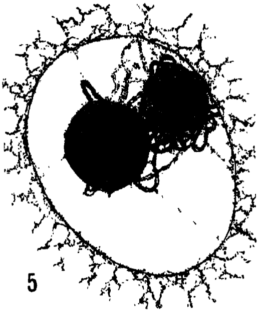
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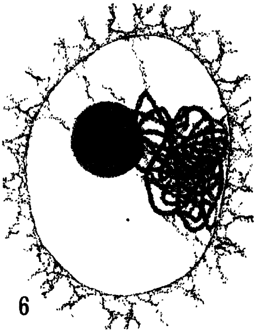
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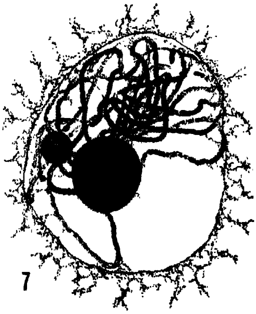
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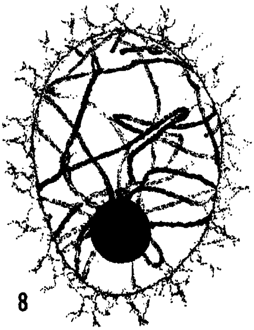
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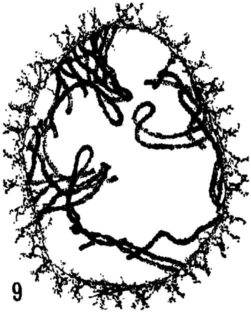
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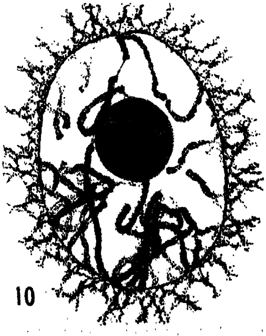
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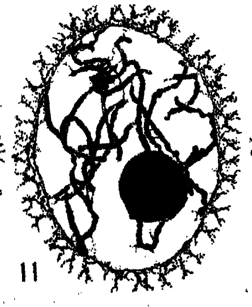
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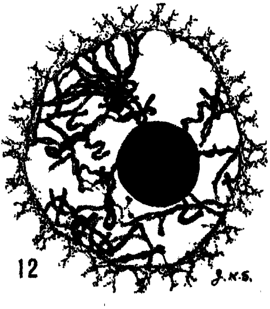
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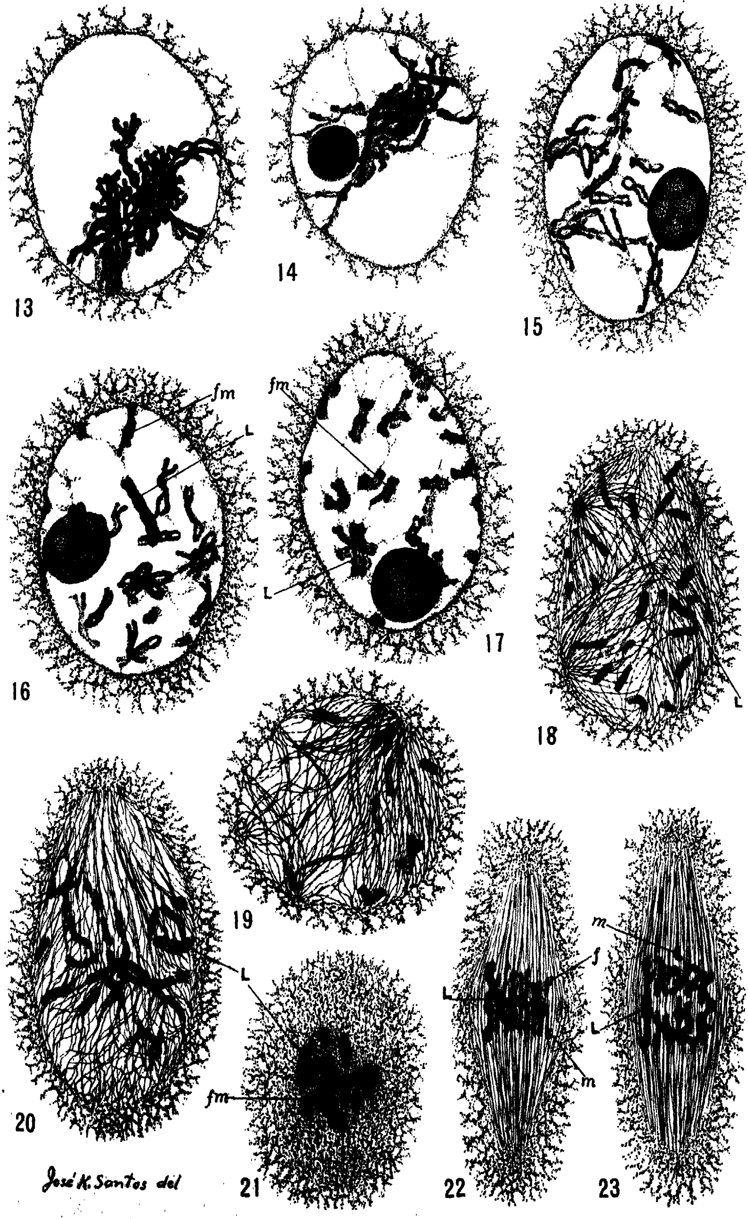

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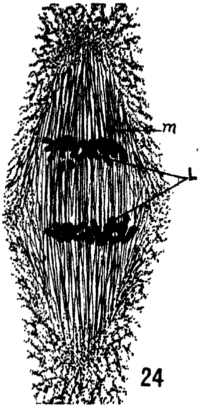

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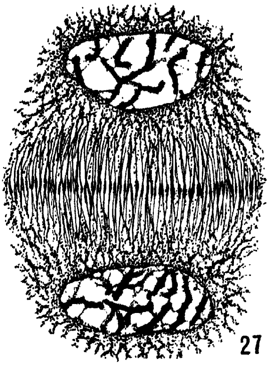

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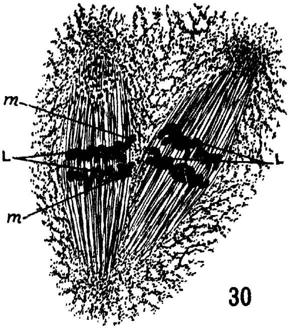

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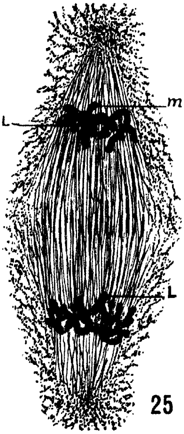

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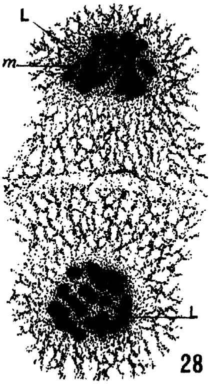

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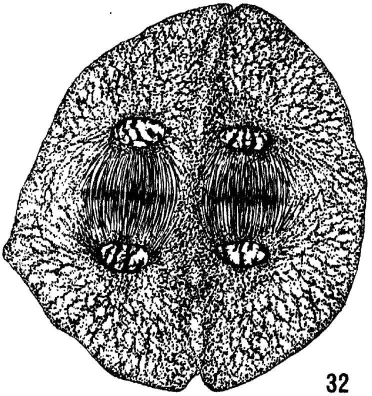

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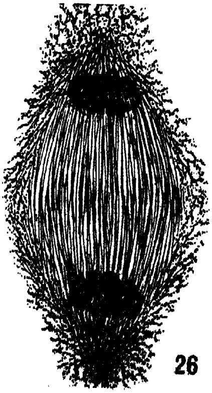

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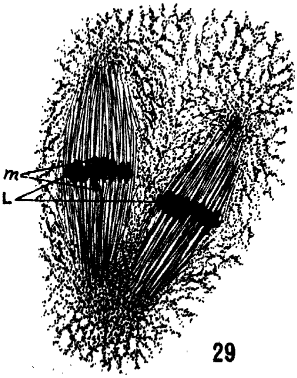

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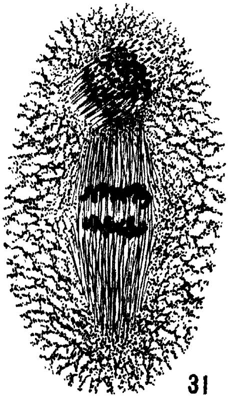

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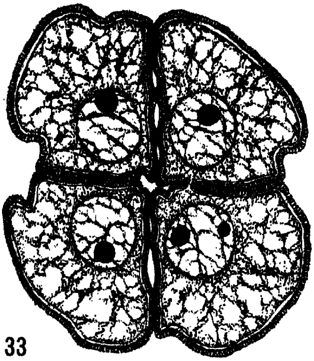

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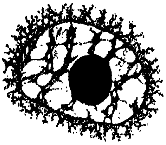

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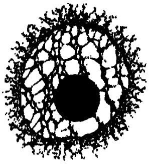

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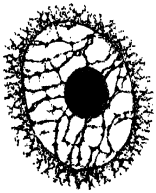

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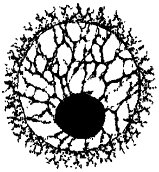

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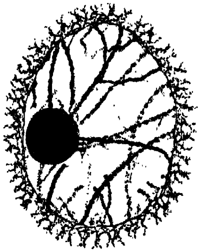

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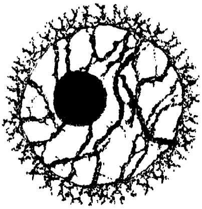

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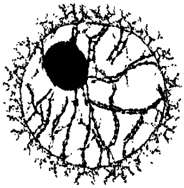

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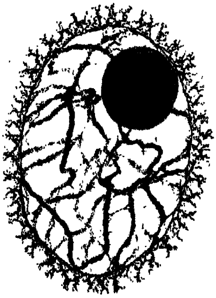

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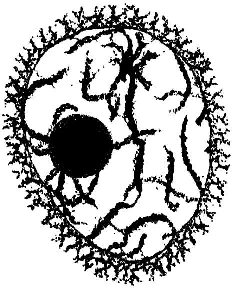

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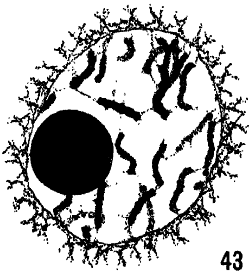

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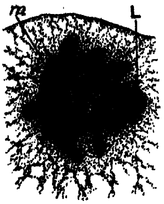

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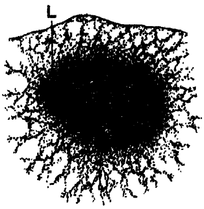

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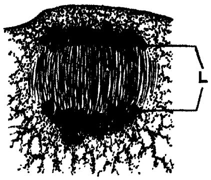

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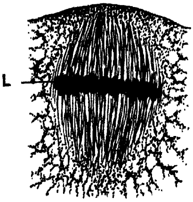

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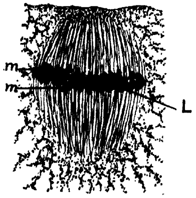

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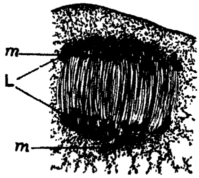

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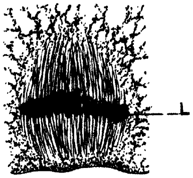

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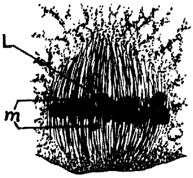

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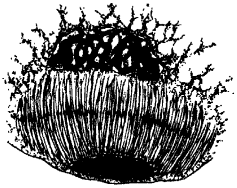

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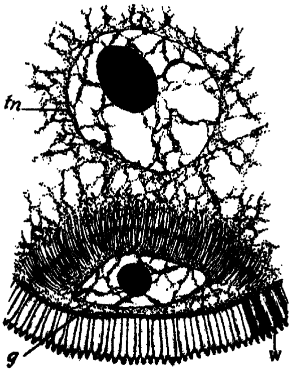

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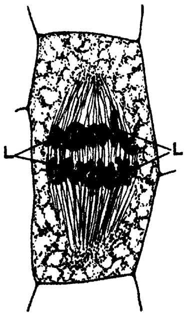

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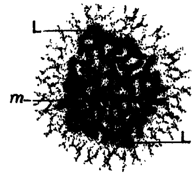

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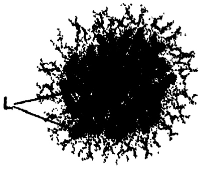

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PROTEIN SYNTHESIS BY PLANTS

I. NITRATE REDUCTION[1]

SOPHIA H. ECKERSON

Introduction

Many workers have studied protein synthesis and some progress has been made, but there are still so many gaps in our knowledge that it is impossible to form a clear idea of the process. There is considerable confusion and some contradictory evidence in the literature of the subject. For instance, the effect of light has been a mooted question from the beginning. One says "Light is necessary"; one "Light is not necessary"; and the next "Is light necessary?" It may be that light is necessary for one of the steps but not for others; or it is possible that light, even though not necessary, may greatly influence the rate at some part of the process. It is hoped that by following the process step by step on the same kind of plants and with the same technique, it may be possible to clear up a few of the much discussed points and thereby add a little to our knowledge of the process as a whole.

In this study of protein synthesis there are three distinct phases which will be considered: (1) reduction of nitrates; (2) synthesis of amino acids; and (3) linking together of amino acids. This paper deals with the first phase.

Material and methods

In a study of protein synthesis one must guard against the possibility of mistaking regenerated proteins for those newly synthesized, and amino acids from the hydrolysis of proteins for newly synthesized amino acids. Such a possibility of confusion is reduced to a minimum by the use of carbohydrate-high tomato plants, grown as described by KRAUS and KRAYBILL (15), as experimental material. They state as follows:

> Plants grown with an abundant supply of nitrogen and then transferred and grown with a very low supply of available nitrogen are very weakly vegeta-

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[1] The work is being carried on at the University of Wisconsin, in cooperation with Professor E. J. KRAUS.

> tive and unfruitful. As compared with the vegetative plants, they are very much lower in moisture and total nitrogen and are lacking in nitrate nitrogen; they are much higher in total dry matter, free-reducing substances, sucrose, and polysaccharides. On supplying nitrate to the soil, such plants . . . . first began active growth at the stem tips. This was associated with a greening of the younger leaves and a very rapid disappearance of the starch grains from the pith cells of the stem, first near the tip and then progressively down the stem to its very base.

It seems that such plants must have all the essential substances and conditions necessary for protein synthesis, except available nitrogen, since new growth begins immediately upon the addition of nitrates. Any amino acids appearing in these low-protein plants soon after the addition of nitrates are almost certainly newly synthesized. This is to be differentiated from the increase in soluble nitrogen in carbohydrate-high plants in the dark (NIGHTINGALE), and from the production of new shoots at the base of carbohydrate-high plants after the tops have been removed (15). Another advantage is that, since there is an abundance of carbohydrates, the plants can be kept in darkness for several days without danger of sugar depletion.

Tomato plants of the Bonny Best variety were grown in rich soil until about 8 inches high, then transferred to quartz sand and grown until typically carbohydrate-high in appearance. The stems were stiff, the younger leaves small, somewhat yellow, and growth at the tip had stopped. The older leaves frequently were purplish in color, due to the presence of anthocyanin. The plants were watered daily with city water, and in addition twice a week with dilute nutrient solution lacking nitrogen.

A microchemical examination of these carbohydrate-high plants was made. Calcium nitrate was then supplied to the sand in five pots (ten plants) and the subsequent chemical changes followed closely by microscopic and microchemical examinations. Frequent examinations were made of both nitrogen-high (carbohydrate-low) and carbohydrate-high (nitrogen-low) plants for comparison. Two weeks later another lot of ten plants was given calcium nitrate. This was continued until there were several series, each at a different stage of vegetative growth.

Microchemical tests

NITRATES.—1. Blue color with diphenylamine in sulphuric acid (0.1 gm. diphenylamine dissolved in 10 cc. sulphuric acid, 3 parts conc. H_2SO_4 to 1 part water). 2. Crystallization as potassium nitrate in sections of the tissue in absolute alcohol on the slide. Identification by polarized light.

NITRITES.—1. Red color with sulphanilic reagent (*a*, sulphanilic acid 0.5 gm., acetic acid (33%) 150 cc.; *b*, alphanaphthylamine 0.1 gm., distilled water 20 cc., acetic acid (33%) 150 cc.). Dissolve the alphanaphthylamine by warming in 20 cc. water. Combine solutions *a* and *b* and keep in a tightly stoppered bottle (FRED 13). 2. Crystallization as silver nitrite.

AMMONIA.—1. Yellow color with Nessler's reagent. 2. Crystallization as ammonium magnesium phosphate.

P_H VALUE.—The Clark and Lubs indicators made up according to directions given by CLARK (10) for aqueous solutions of thė alkali salts were used.

RESULTS

BEFORE ADDITION OF NITRATES.—When the carbohydrate-high plants were ready for experimentation they contained an abundance of glucose, some fructose, and a little sucrose. In the stem pith and endodermal and cortical cells were filled with starch. In the leaves the parenchyma cells, even of the very youngest leaves, were packed with starch grains. The tissue gave no reaction for nitrates, nitrites, or ammonia. No amino acids could be detected.

AFTER ADDITION OF NITRATES.—Within twenty-four hours nitrates were present in all parts of the plants. The tops of a few plants gave a slight reaction for nitrites. There was no reaction for ammonia.

Within thirty-six hours all the plants had considerable nitrite, strictly localized in the following regions: *a*, in the cortical cells at the tips of the stems; *b*, in the cortical cells of the petioles and near the veins of the younger leaves; *c*, in the stem (at the nodes), in the cortical cells near the phloem, and in the phloem parenchyma. In all these regions there was a trace of ammonia. Within forty-eight hours there was slightly less nitrite but more ammonia.

A few crystals of asparagin appeared on putting sections in absolute alcohol. At this time there was a slight but definite decrease in the amount of starch in the cortical cells at the tips of the plants and from the youngest leaves. Within three to five days there was very much less nitrite, a little ammonia, but a great increase in amino acids. Succinic acid and malic acid were now present, as well as the following amino acids: aspartic acid, asparagin, alanine, leucine, cystine, histidine, and at least two not yet identified. Simultaneously with this appearance of amino acids starch was disappearing and the chloroplasts of the younger leaves were becoming greener. Externally the plants showed signs of new growth at the tips. These and further developments are described fully by KRAUS and KRAYBILL (15).

> Within three days following such transfer (from no nitrogen to available nitrogen) the beginning of the disappearance of the starch grains from the center pith cells and cortical cells at the tips of the plants was very noticeable. Successive examinations as growth progressed showed an active terminal elongation which contained no storage starch except in the starch sheath, an active development of secondary xylem in the older portion of the stem, and a very rapid, progressive, and finally complete disappearance of the starch from the pith and xylem parenchyma and also the cortical cells even down to the bases of the stems, where it was the last to disappear.

Beyond the first five to ten days after nitrates had been supplied there was only a little nitrite at any time. There were even occasional times when none could be detected in a few of the plants. Ammonia was always present in small amounts, from a trace to a little more. Amino acids apparently increased in amount up to about the third week.

P_H VALUES.—The P_H values for the different tissues of tomato plants which had just been supplied with nitrates proved to be very interesting. In a cross-section of the stem the values were approximately: center pith cells 5.2–5.0, xylem cells 4.6–4.4, phloem parenchyma and inner cortical cells 7.2–7.6. In the younger leaves the values were approximately: xylem and adjacent parenchyma cells and cortical cells 5.8–5.6, phloem parenchyma and nearby cortical cells 7.2 to 7.4 or 7.6; that is, the cells where starch was being hydrolized were slightly acid, while the cells where nitrate was being reduced were slightly alkaline.

Reduction of nitrates by plant extract

PLANT POWDER.—The leaves of nitrogen-high tomato plants were removed and kept separate from the stems, which were separated into nodes and internodes. These were cut into small pieces and ground fine in a meat grinder. Each lot was squeezed through folded cheesecloth. The juice was then treated with 30 per cent alcohol, filtered, and the filtrate precipitated with 95 per cent alcohol. The precipitate was taken up in water, again precipitated with 95 per cent alcohol, then once more taken up in water and precipitated with 95 per cent alcohol. This final precipitate was dried on the filter paper, then the dry snow-white powder was scraped off and kept in stoppered vials, in darkness.

TABLE I

REDUCTION OF NITRATES IN ALKALINE SOLUTION, ACCELERATED BY ADDITION OF PLANT POWDER; 10 CC. 2 PER CENT KNO_3 PLUS CARBOHYDRATE WITH OR WITHOUT $NaHCO_3$ WITH OR WITHOUT 0.1 GM. LEAF POWDER PLUS TOLUOL; 20 HOURS IN DARKNESS AT 50° C.

Carbohydrate	With or without Na_2CO_3	P_H value	With or without plant powder	Nitrite (mg. per 100 cc.)
1 cc. 10 per cent				
1. acetaldehyde	Plus	7.2	Leaf	2.0
2. acetaldehyde	Minus	5.2	Leaf	Trace(?)
3. acetaldehyde	Plus	7.2	Leaf (boiled 2 minutes)	2.5
4. acetaldehyde	Plus	7.2	Internodes	2.5
5. acetaldehyde	Plus	7.2	Nodes	1.6
6. acetaldehyde	Plus	7.2	Minus	0.7
7. fructose	Plus	7.2	Leaf	1.6
8. glucose	Plus	7.2	Leaf	1.0
9. acetaldehyde	Plus	7.2	1 gm. leaf	5.0

It was found that small amounts of this plant powder accelerated the rate of reduction when added to an alkaline solution containing nitrates and some easily oxidizable carbohydrate. There was very little or no reduction in acid solution. The results given in table I are typical. In the series the amount of plant powder used in each case was 0.1 gm. for 10 cc. potassium nitrate. If this amount was increased the rate of reduction increased, but not in proportion, as shown in 9 at the foot of the table.

Many series of experiments were made to determine the most favorable hydrogen-ion concentration. One of the later series is given in table II. It will be more profitable to discuss these results after the presentation of data concerning experiments with plant juice.

PLANT JUICE.—The juice of nitrogen-high and of carbohydrate-high tomato plants was extracted by grinding up the tissues and squeezing through folded cheesecloth. Toluol was added at once to prevent bacterial action.

This fresh juice had an exceedingly high reducing activity. The juice from nitrogen-high plants contained an abundance of both nitrates and sugars. It was only necessary, therefore, to reduce the hydrogen-ion concentration (P_H 6.4) to approximately P_H 7.6 and reduction began at once. The carbohydrate-high plants contained an abundance of sugar but no nitrate. The juice of such plants gave no reaction for nitrites until after nitrate had been added and the whole made slightly alkaline. At a P_H value of 7.6 the reducing activity was even greater than that of the juice from nitrogen-high plants. Table III shows the rate of reduction at 50° C.

TABLE II

EFFECT OF HYDROGEN-ION CONCENTRATION ON REDUCTION OF NITRATES; 10 CC. 2 PER CENT KNO_3, CARBOHYDRATE, N/10 NaOH, AND 0.1 GM. LEAF POWDER WITH TOLUOL; IN DARKNESS AT 50° C.

Carbohydrate	N/10 NaOH (cc.)	P_H value	Nitrite (mg. per 100 cc.) 24 hours	Nitrite (mg. per 100 cc.) 36 hours
1 cc. 10 per cent				
1. acetaldehyde.........	0.6	7.0*	1.0	1.5
2. acetaldehyde.........	0.75	8.4*	3.3	7.5
3. acetaldehyde.........	0.8	9.2	1.8	1.0
4. 0.1 gm. fructose.......	0.2	8.4*	6.5	10.0
5. 0.1 gm. fructose.......	0.3	9.2	2.5	2.0

* At the end of 36 hours the P_H value of 1 was 6.8; of 2, 7.6; and of 4, 7.4.

Boiling the juice considerably increased the acidity. Such acid juice reduced nitrates to nitrites only to a very slight extent, or not at all. In every case when the hydrogen-ion concentration was decreased to a P_H value of approximately 7.6, however, the reducing activity was as great as before heating. The longer the juice was boiled the more acid it became, and, naturally, the more sodium was required to bring it to the P_H value 7.6. This is shown in table IV.

HYDROGEN-ION CONCENTRATION.—The most favorable P_H value for reduction of nitrates by plant juice was approximately 7.6. This value was maintained nearly constant (7.6–7.4) throughout the experiment, probably owing to the presence in the juice of inorganic salts which acted as buffers. On the other hand, solutions of the plant powder containing little inorganic salt quickly became more acid on oxidation of the aldehyde or sugars. When the P_H value at the start was 7.6, in a short time it was 7.2 or 7.0 when

TABLE III

REDUCTION OF NITRATES BY JUICE OF NITROGEN-HIGH (N+) AND CARBO-HYDRATE-HIGH (C+) TOMATO PLANTS; IN DARKNESS AT 50° C.; TOLUOL ADDED IN ALL CASES

PLANT JUICE	N/10 NAOH PER 10 CC.	P_H VALUE	NITRITE (MG. PER 100 CC.)		AMMONIA 20 HOURS
			3 hours	20 hours	
1. 20 cc. N+	0.5	7.6	2.5	50.0	Trace
2. 20 cc. N+	0.0	6.4	1.0	5.0	None
3. 10 cc. C+	0.2	7.6	0.0	Trace?	None
4. 10 cc. C+ and 10 cc. 2 per cent KNO_3	0.3	7.6	3.3	40.0	Trace
5. 10 cc. C+ and 10 cc. 4 per cent KNO_3	0.4	7.6	3.3	60.0	Trace
6. 10 cc. C+ boiled 10 minutes and 10 cc. 4 per cent KNO_3	0.0	3.0	0.0	00.00	None
7. 10 cc. C+ boiled 10 minutes and 10 cc. 4 per cent KNO_3	1.2	7.6	5.0	50.0	Some

TABLE IV

INCREASE OF ACIDITY OF TOMATO PLANT JUICE ON HEATING; 10 CC. CARBOHYDRATE-HIGH (C+) JUICE, BOILED, AND N/10 NaOH TO BRING TO P_H 7.6

C+ juice boiled (minutes)	N/10 NaOH per 10 cc.	P_H value
1. 0.0	0.20	7.6
2. 0.5	0.30	7.6
3. 2.0	0.75	7.6
4. 5.0	0.90	7.6
5. 10.0	1.20	7.6
6. 15.0	1.50	7.6

reduction was greatly decreased. The greatest reduction, however, was obtained when the value at the start was 8.4 and at the end of 20 hours 7.6–7.4. It seems, therefore, that the most favorable P_H value for reduction of nitrates by plant powder as well as by plant juice must be near 7.6. It will be remembered that the P_H value of the cells of the living plant when reduction of nitrates was taking place was usually 7.4 to 7.6, although occasionally 7.2 or 7.8.

LIGHT.—In all of the earlier experiments check series were run in both light and darkness. I was never able to detect any influence of light on either the rate or the amount of reduction of nitrates by plant extract (powder or juice). In the living plant there is reduction of nitrates in darkness, and for the first two or three days, seemingly at about the same rate as in light. After that differences appear, the exact nature of which is not yet clear. This is being studied further in connection with amino acid synthesis.

OXYGEN.—It was early found that any shutting out of air from the surface of the reacting solutions greatly decreased the amount of reduction, indicating that some free oxygen is required. In all of the experiments described in this paper the reacting solutions were in 50 cc. flasks, thus allowing free access of air to the relatively large surface.

PLANT POWDER.—Since the activity of the plant juice far exceeded that of the plant powder, much must have been lost somewhere in the process of preparing the powder. It was found to be in the final precipitation with 95 per cent alcohol. An attempt was made to prepare a more active powder by eliminating this step. Carbohydrate-high tomato plant juice was squeezed through fourfold cheesecloth, precipitated with 30 per cent alcohol, and filtered. After evaporation of the alcohol this filtrate was highly active. It was evaporated to dryness in 10 cc. lots at 50° C., and the reducing activity tested. It is not known how long this dry material would retain its activity. There was no loss at the end of five days, when the last lot was tested. A single experiment will suffice to show the activity: C+ juice after precipitation with 30 per cent alcohol. Ten cc. filtrate evaporated to dryness at 50° C., taken up in 10 cc. water+0.2 gm. KNO_3+0.1 gm. fructose+0.6 cc. N/10

NaOH (to give P_H 7.6) in 20 hours at 50° C. gave 50.0 mg. nitrite per 100 cc.

Discussion

REDUCASES.—In 1886 REY-PAILHADE discovered an enzyme in yeast that reduces sulphur by adding hydrogen. This he named philothion. Later, POZZI-ESCOT (19) found that philothion also reduces nitrates to nitrites. In 1902 SCHARDINGER (20) found an enzyme in fresh milk which reduces methylene blue to its leucobase. Later, BACH (2) found that the Schardinger enzyme also greatly accelerated the reduction of nitrates to nitrites by an aldehyde. BACH (3) also found that an enzyme could be extracted from calf liver which accelerates the reduction of nitrates in the presence of acetaldehyde. So far as I have been able to find, there are only three workers who have made a study of the reduction of nitrates by reducases obtained from plants: POZZI-ESCOT (19) from burdock stems; KASTLE and ELVOVE (14) from potato tubers and etiolated sprouts, and egg plant fruit; and BACH (5) from potato tubers. BALY, HEILBRON, and HUDSON (6), and BALY, HEILBRON, and STERN (7) have studied the reduction of nitrates and the simultaneous oxidation of formaldehyde in ultra violet light. Although these BALY papers are valuable, they should be read with caution, as they contain much that is purely hypothetical. Finally, there is a series of papers by BAUDISCH (8, 9) on the reduction of nitrates in the presence of glucose and traces of iron.

REACTION OF MEDIUM.—The reaction of plant extracts depends largely upon the plant, but also upon the method of extraction and subsequent treatment. The P_H value of nitrogen-high tomato plant juice immediately after being squeezed through cheesecloth was 6.4, after filtration through asbestos 6.0, after precipitation with 30 per cent alcohol 5.8. To bring all to the same P_H value different amounts of hydroxide or of carbonate must be added. In previous work no exact determination of the acidity or alkalinity of the plant extracts was made. For this reason it is difficult to interpret some of the data. POZZI-ESCOT (19) found that the solution of the yeast enzyme (philothion) had a slight acid reaction. The addition of traces of alkali gave a very active solution. The "addition of any considerable amount of a mineral acid or a strong organic acid (acetic) acts as a strong paralyzer." Of the salts, "the most energetic paralyzers are those with an acid reaction." This indicates that this reducase is most active in a slightly alkaline solution, which agrees with the present work with tomato reducase. KASTLE and ELVOVE (14), working with unfiltered potato extract, state that probably a slightly acid medium is best, as when sodium hydroxide or lime water are added "to neutralize" there is no reduction. They used 1 cc. N/10 NaOH to 10 cc. potato extract, and 2 cc. of 50 per cent KNO_3. Unless the potato extract was more acid than tomato plant juice, this would seem to be too much alkali. BACH (3) states that the addition of sodium bicarbonate (1 gm. to 100 cc.) had no effect on the reduction of nitrates in the system, that is, reducase (from calf liver or milk) plus $NaNO_3$ plus acetaldehyde. BACH apparently was working with neutral solutions, as twice in the process of extracting reducase from calf liver he treated with sodium bicarbonate, then neutralized with acetic acid.

Without exception, every one who has studied the reducing enzymes states that heating (90–100° C.) for 3–5 minutes destroys the activity. Heating plant juice increases the acidity. Tomato plant extract was as active in reducing nitrates after boiling as before, provided the solution was made slightly alkaline.

POZZI-ESCOT compared catalase with philothion, and found them similar in several characteristics. Both have the ability to split off oxygen from hydrogen peroxide and both reduce nitrates. He concludes that catalase is a reducase. It has been shown (1, 11, 16) that catalase is more active, in splitting off oxygen from hydrogen peroxide, in an alkaline solution.

Oxidations and reductions go on at the same time. If one substance is reduced another must be oxidized. DAKIN (12) considers that "reductions and oxidations are two expressions of one process, dehydration." He prefers the term dehydrase to reducase. For the process of reduction, and accompanying oxidation, some easily oxidizable substance is necessary, also free oxygen. The early workers added an aldehyde to solutions containing nitrate and animal or plant extract. BACH (3) found that acetaldehyde was better than formaldehyde. BALY, HEILBRON, and HUDSON (6), in their work on the reduction of nitrates in ultra violet light, used formaldehyde. They think that nitrates are reduced in the light by activated formaldehyde in green leaves. In their opinion newly synthesized formaldehyde is in a highly active state.

This theory could scarcely be considered in the case of carbohydrate-high tomato plants. Here it is the excess stored carbohydrates that is used up rapidly when nitrates are present. Fructose and glucose are oxidized, some perhaps in increased respiration, but much of them to form amino acids. As rapidly as fructose and glucose are used, more starch is hydrolyzed. In the experiments with tomato plant powder, therefore, the effectiveness of fructose and glucose was tested in comparison with acetaldehyde. In the early experiments, where the reaction was about neutral, the order according to the amount of nitrite produced was acetaldehyde>fructose>glucose, but at a P_H value of 8.4–7.6 the order was fructose>glucose>acetaldehyde. BAUDISCH (9) found glucose effective in the reduction of nitrites. He states that "the smallest trace of an iron salt is sufficient to reduce a large amount of nitrite on warming with glucose in weakly alkaline solution." That oxygen also is necessary has been shown by several workers. DAKIN states that methylene blue could not be decolorized by the dehydrase (reducase) of milk in an atmosphere of hydrogen. BAUDISCH "found that ferrous bicarbonate, or hydroxide, in the absence of oxygen even at the temperature of boiling water does not attack alkali nitrates to the slightest extent; the presence of oxygen, however, brings about immediate reduction to nitrite, and there is a direct relation between the amount of oxygen dissolved in the water and the amount of nitrite formed." There was no reduction of nitrates by tomato plant extract if the solution was in small vessels filled, for the exclusion of air, and stoppered.

BALY and associates find that in their experiments reduction of nitrates takes place only in ultra violet light, but they think light is necessary only to activate the formaldehyde, that the reduction itself is not a photochemical process. BAUDISCH (9) found that "in the case of cholera bacteria the reduction proceeds just as rapidly in the dark as it does in the light." Here he found the reduction of nitrates to nitrites is in direct relation to the oxygen respiration of the bacteria and to their iron content. The rate of reduction of nitrates by tomato plant extract in darkness was the same as in light. There was reduction of nitrates by tomato plants in darkness, and for the first two or three days, at least, it seemed to go on at about the same rate as in the light.

From the foregoing results there seems but little question that there is in tomato plants an active substance which, on being added to an alkaline solution of potassium nitrate containing fructose or glucose, causes rapid reduction of nitrates to nitrites, and a slower reduction of nitrites to ammonia. The nature of this substance is not known. Is it an enzyme of organic nature, or is it an inorganic substance? It is not impossible that it may be iron, and the chemical process may be that suggested by BAUDISCH (9). If it were iron, however, judging from the work of NEUBERG (17, 18) on the catalytic action of sunlight in the presence of small amounts of iron compounds and from my own work with light-sensitive seeds, I should expect an increased activity in light. It may be possible in the future to determine the nature of the active substance.

Summary

1. Reduction of nitrates to nitrites and ammonia was obtained by tomato plant extract in slightly alkaline solution in the presence of fructose or glucose and some free oxygen in darkness as well as in light.

2. The reduction was most active in solutions having a P_H value of approximately 7.6.

3. The expressed juice of nitrogen-high tomato plants (containing both nitrates and sugar in abundance), after adding N/10 NaOH to bring to a P_H value 7.6, gave a very rapid reduction of nitrates to nitrites at 50° C.

4. The expressed juice of carbohydrate-high tomato plants (containing much sugar but no nitrates), brought to P_H 7.6, gave no reaction for nitrites after 20 hours at 50° C. Upon addition of nitrates there was even more rapid reduction than by the nitrogen-high juice.

5. Tomato plant juice boiled and brought to a P_H value 7.6 reduced nitrates as rapidly as the unheated juice.

6. The rate and amount of reduction of nitrates to nitrites by tomato plant extract were exactly the same in darkness as in light.

7. Probably the rate of reduction of nitrates to nitrites by carbohydrate-high tomato plants in darkness was the same as in light for the first few days. Then disturbing differences appeared which are not yet understood.

8. In carbohydrate-high tomato plants the reduction of nitrates was localized in the following regions: at the stem tip just behind the growing region; in the leaf cells, especially near the phloem and in the cortical cells of the petioles; in the stem, especially near the nodes, in the phloem parenchyma, and in cells in the cortex near the phloem.

9. All these regions were slightly alkaline in reaction. The pith cells were acid. In the carbohydrate-high plants before nitrate was supplied there was less difference. The pith cells were slightly acid while the phloem parenchyma was about neutral in reaction.

10. Amino acids (newly synthesized) appeared at the nodes and in petioles and blades of young leaves and just behind the stem tips of carbohydrate-high tomato plants three or four days after nitrate had been supplied to the soil. The amino acids were aspartic acid, asparagin, alanine, leucine, cystine, and histidine.

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FEMALE GAMETOPHYTE OF MICROCYCAS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 318

LILLIAN GRACE REYNOLDS

(WITH PLATES XXVIII, XXIX AND SIX FIGURES)

Microcycas calocoma is an endemic genus with a single species, occurring in mountainous regions of Pinar del Rio in western Cuba. It was first described by MIQUEL (14), whose description was amplified by DE CANDOLLE (9). In 1907 CALDWELL and BAKER (2) published the first adequate account, and a few months later CALDWELL (1) gave the first satisfactory description of the genus, and the first account of its life history. The name *Microcycas* proved to be a misnomer, for it is a tree, only two other genera of cycads having taller species.

The material for this paper was collected by Professors CALDWELL and CHAMBERLAIN during the years 1907–1911, but at different seasons. Professor CHAMBERLAIN collected still other material in 1914, and again in 1922. The sections from which the study was made were prepared by CALDWELL, Miss PACE, YAMANOUCHI, and myself. The earlier sections were stained in gentian violet, the others in Haidenhain's iron-alum haematoxylin, and to those most recently made, gold orange was added to bring out the cell walls. I am under great obligation to Professor CHAMBERLAIN for suggestions and criticisms during the progress of this investigation, and to P. J. SEDGWICK for valuable assistance in the making of the photomicrographs.

Development of endosperm

YOUNG GAMETOPHYTE.—The youngest stage found in the development of the female gametophyte of *Microcycas* shows free nuclear division in the megaspore (fig. 7). The material, although fixed in August, is not young enough to show the megaspore mother cell or the megaspores. The ovules are about 2 mm. long (fig. 8), and the gametophyte within is about half that length. The gametophytes of this size vary somewhat in the number of free nuclei, the lowest number noted being 64, the next 256, all in early anaphase, but not suitable for counting the individual chromosomes; while in the gametophyte in the other ovule on this same sporophyll there has been one more mitosis, so that the number theoretically is 512. In another ovule of the same date, there has been one more division, so that the theoretical number of nuclei is 1024, but at this stage some of the nuclei are almost sure not to divide, so that the theoretical number is seldom or never reached. Considering the size of the gametophyte, there are probably no more nuclear divisions before the walls come in.

The early stages in wall formation, showing the first walls of the gametophyte striking in toward the center, might have been found in September ovules had they been available. The gametophyte in early October is about 3 mm. long, and shows that the peripheral nuclei are completely inclosed within walls. The gametophytes examined are badly shrunken, probably on account of the killing agent, and it is difficult to determine whether the nuclei in the center are free, or whether their walls are so delicate that they cannot be identified. In material dating from the middle of October to November, the female gametophyte is completely cellular (fig. 9).

MATURE GAMETOPHYTE.—From about November 22 to early December, the archegonial initials appear. In CHAMBERLAIN'S most recently collected material, the archegonial initials appeared in September, which is two months earlier than in ovules previously examined. This may be due to variance in the seasons of different years. The length of the ovule by this time is approximately 1 cm., and the length of the gametophyte 4–5 mm. Transverse sections show the cells arranged in regular rows converging toward the center (fig. 11). The peripheral cells are smaller than the interior cells, and are practically isodiametric. These peripheral cells are the result of many transverse and vertical divisions of the original cell. Those that are nearest the original walls apparently do not divide as frequently, and often have less cytoplasm than those in the middle. From these last are differentiated the archegonial initials (fig. 13).

Although not evident in ovules before this date, there now appears a "median cleft" (CALDWELL 1), or "closure" (Miss CAROTHERS 3), or suture. Cleft is an unfortunate term, since it implies the separation of parts once united, which is just the opposite of what is really the case. Closure is more apt, since it expresses the closing up of the central vacuole by the centripetal growth of the surrounding cells. Suture suits exactly the appearance of this median line as seen in stained sections. According to WEBSTER'S dictionary a suture is "the line or seam, formed by the union of two adjacent margins." Absolutely median sections of the mature gametophyte show the cells arranged in rows converging toward this suture (fig. 15). If the sections are not median, only the cells bordering on the original cell walls of the gametophyte appear in radiating rows. The others, being cut obliquely and transversely, appear as irregular patches of round cells bounded by cells in regular rows (fig. 16). Also, there are cracks or clefts connecting this suture with the exterior. These occur along the original walls, separating the progeny of one original cell from that of another. The extent of the progeny of each cell is visible on the surface of the gametophyte as a slight swelling, making the entire surface appear papillate.

The young cells of the gametophyte are uninucleate. In a mature gametophyte there may occasionally occur cells containing two nuclei, due to the breaking down of the walls between the two cells.

As a rule, the number of chromosomes is twelve, the number found in all the cycads which have been investigated; however, in two cases fourteen chromosomes were counted.

Nutrition of gametophyte

ENDOSPERM JACKET.—Around the youngest gametophyte examined there was a layer of cells about four or more cells thick (fig. 8). These cells are smaller than the cells of the integument, but similar to those of the nucellus in size of cell and size of nucleus. In other gametophytes of about the same age, this layer of four cells is differentiated into a layer two cells thick of plump cells immediately surrounding the gametophyte, and a layer outside of that of flattened cells fitting loosely together. There is a sharp line of demarcation between these latter and the cells of the nucellus. The inside layer of cells is to become the nutritive jacket of the gametophyte. These cells are large, flat, and full of cytoplasm in which are imbedded large nuclei, nearly half the diameter of the cells. By October the walls are no longer thin, having thickened appreciably, while the cells themselves are still plump (fig. 10). The nutritive jacket cells around the gametophyte in November have very thick walls, on the inside of which are granules laid closely together (fig. 13). The nuclei are not so large in proportion to the size of the cell as in the preceding stages. In gametophytes a few days older, the cells of the jacket layer have become flattened, the walls are very thick, the granules are prominent, and the nuclei are much smaller. By the middle of December the nutritive jacket is practically all gone (fig. 17), only such vestiges as pieces of the granular cell walls remaining. At this time, too, the cells of the nucellus immediately above the archegonia are gone, and those above these are represented only by their cell walls.

CONTRIBUTION FROM SPOROPHYTE.—The vascular bundles belonging to the inner fleshy layer often branch just below its union with the nucellus, one strand continuing in the inner fleshy layer, and the other extending into the free portion of the nucellus, thus contributing directly to the nourishment of the gametophyte. Spiral elements in the bundle can be distinguished among the elongated, but as yet unmodified procambial cells as early as the archegonial initial stage. In the mature seed, when the inner fleshy layer of the integument and the nucellus have become papery, these branches can be seen like frayed ends extending 1.5–2 mm. into the almost transparent nucellus.

MEGASPORE MEMBRANE.—Meanwhile the wall of the megaspore becomes much modified, and is a noticeable membrane adhering closely to the gametophyte. The diameter of the megaspore membrane is smallest during the free nuclear division of the gametophyte (fig. 7), when it is approximately 0.5 μ wide; and 3 μ wide (its greatest width) about three months later when the gametophyte has just become cellular (fig. 9). From this time the archegonia are developing very rapidly, and the strain of the expanding tissue stretches the membrane at the micropylar end until it measures only 2.5 μ during November (fig. 13), 1.5 μ during early December, and only 1 μ from the middle of December until the end of March, or just before fertilization. A measurement made of the membrane from the side of the gametophyte at this date is also 1 μ, as if the strain were not localized at the micropylar end, but felt all over. This is not inexplicable when the size of the mature gametophyte is considered.

In one of the ovules dated October 31, two gametophytes had developed (fig. 12). The centripetal growth of cell walls was not yet complete. Although both are inclosed by a common nutritive jacket, each is surrounded by an entire megaspore membrane, indicating individual origins from two megaspores, the only case of this kind yet reported for cycads.

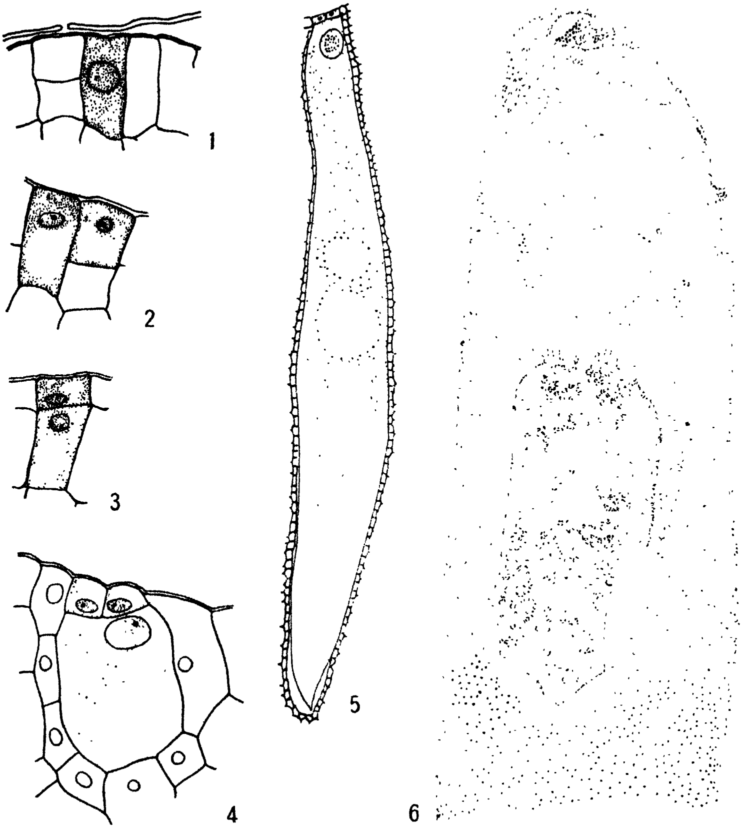
Archegonia

DISTRIBUTION.—In the young gametophyte it is difficult to determine which cells should be regarded as archegonial initials, because all over the surface of the gametophyte are groups of cells not more than three or four cells apart, each cell containing a large nucleus and an abundance of cytoplasm (fig. 14). That these all may be potential archegonia is shown by the fact that in the mature gametophyte as many as four, five, or six archegonial groups may be seen scattered on the surface, in addition to the large group at the micropylar end. Of these groups, however, the only one to progress so far as to have the ventral canal nucleus division occur in its archegonia is that group which is beneath the micropyle. The archegonia of the remaining groups begin to disorganize by the time the central cell has enlarged to three or four times its original size.

Not only are some archegonia scattered over the surface of the gametophyte, but occasionally they develop entirely inside, in groups or isolated. Generally they are at a considerable distance from the micropylar group, but do occur within this group itself, although at right angles to the other archegonia. In such archegonia as have been examined, the large central cell nucleus is imbedded in comparatively dense cytoplasm at one end, while the other end is very vacuolate, as in ordinary archegonia. The neck cells were indistinguishable from the other cells of the gametophyte. That any of these archegonia definitely open on to sinuses seems improbable, but this could not positively be ascertained.

It would seem that the nourishment of the gametophyte might be one of the factors, or might be the particular factor, influencing the localization of the best developed archegonia at the micropylar end of the gametophyte. Not much food enters the gametophyte from the basal end of the ovule. The vascular bundles entering the inner fleshy layer branch very freely, and practically all of these branches terminate near the micropylar end of the gametophyte, so that this vascular system contributes more nourishment to this end of the gametophyte than to the basal. Then, also, the contents of the cells liberated by the formation and subsequent enlargement of the pollen chamber in the nucellus are probably absorbed, just as the cell contents are absorbed from the gametophyte jacket cells. Since this takes place at the micropylar end, there would be an increase in the food supply at this end of the gametophyte, thereby promoting the more rapid development of the archegonia here, and retarding and finally eliminating the archegonia scattered elsewhere.

DEVELOPMENT.—The first stage in the development of the archegonium is the differentiation of the archegonium initial from the other cells of the gametophyte. At first the initial is simply a cell better stocked with food and with a larger nucleus than the neighboring cells (fig. 1); then a vacuole appears below the nucleus and enlarges as the cell enlarges, the nucleus remaining at the top (fig. 2). The cytoplasm is likely now to look rather scanty in comparison with the size of the cell, because the increase in the mass of the cytoplasm does not keep pace with the increase in the size of the cell. The nucleus then divides to form the central cell and the primary neck cell (fig. 3), which probably divides almost immediately to form the two-celled neck (fig. 4). Within the next week the central cell enlarges immensely, becoming very vacuolate in the process (fig. 5). In early December, when the gametophyte is about 1 cm. in length, the archegonium is about twenty-four times the length of the original initial. A month later the vacuoles in the cytoplasm vary in size from very large ones in the basal end of the archegonium to very small ones near the neck, while in the cytoplasm surrounding the nucleus there are none at all. As the archegonium matures, the density of its cytoplasm increases by the concentration in the central cell of food received from the archegonial jacket and neighboring cells. At the time the ventral canal



FIGS. 1–6.—Development of archegonium of *Microcycas calocoma:* fig. 1, archegonium initial; fig. 2, archegonium initial with vacuole in base before cutting off of neck cell; fig. 3, archegonium initial divided, giving rise to primary neck cell and central cell; fig. 4, primary neck cell divided to form two neck cells; fig. 5, central cell much elongated before division to form egg; fig. 6, ventral canal nucleus and egg nucleus; note that in this case a faint wall has been laid down on the spindle fibers and a portion of it has been dragged down into the cytoplasm of the egg; figs. 1–4, 6, ×238; fig. 5, ×52.

nucleus is cut off, the cytoplasm appears to be very solid and without vacuoles.

VENTRAL CANAL NUCLEUS.—The division of the nucleus of the central cell to form the ventral canal nucleus and the egg nucleus takes place late in February (fig. 6). In one of the sections observed the division is complete, with the very much enlarged egg nucleus near the middle of the egg. Both nuclei have spindle fibers attached to them; and cutting off the ventral canal nucleus and its cytoplasm from the egg proper is a very faint wall developed on spindle fibers (fig. 6). A fragment of this wall with its fibers has been dragged down into the egg, and shows very clearly that what appeared to be one wall is really two walls. Ordinarily the ventral canal cell is not separated from the egg by a wall. This is the only cycad in which even a trace of a wall has been observed. As to whether it disorganizes or is permanent, there is at hand no material old enough to determine.

NUTRITION OF EGG.—By the time the ventral canal nucleus is formed, the egg is inclosed in a membrane perforated by haustoria which are protrusions of egg cytoplasm. These haustoria pass through the egg membrane in groups of two and three or more (fig. 18). The egg haustoria penetrate the cells of the archegonial jacket and through them the contents of these cells pass into the egg. Finally, these cells are practically exhausted, the cells being empty, scattered, flattened, and otherwise distorted.

The beginning of the archegonial jacket is barely recognizable when the primary neck cell divides to form two neck cells. Thereafter it develops in such a manner as to make it difficult, when looking at a micropylar group of archegonia, to determine whether it is individual for each archegonium, or a region in which the archegonia are imbedded. When, as it sometimes happens, however, one archegonium develops so as to project itself into another, there is always between the two central cells a single layer of cells, the archegonial jacket. Although present, the archegonial jacket in *Microcycas* is not as strongly developed, and does not last as long as in other cycads, being very much exhausted by the latter part of December.

ANOMALOUS ARCHEGONIA.—Numbers of the archegonial initials at the periphery of the micropylar group and scattered over the remaining surface of the gametophyte develop into abnormal archegonia. Some of these anomalous archegonia are multinucleate. This condition, as indicated by different stages found on sectioning, may arise in two ways: either by the division of the central cell nucleus before the greatest elongation of the archegonium and the subsequent cutting off of the ventral canal nucleus (figs. 21–23); or by fragmentation of the central cell nucleus at a period just before the separation of the ventral canal nucleus from the egg nucleus by cutting off of amoeboid arms (fig. 19).

Another variation in archegonia is in the number of neck cells. In one case it appears that the primary neck cell has divided transversely, and in another case, that each of the cells has divided vertically, forming two tiers of neck cells, two cells in each tier (fig. 21).

Generally the archegonia on the sides of the gametophyte are grouped without a definite archegonial jacket. These archegonial complexes probably originate from a group of adjacent archegonial initials, all of which develop, permitting no jacket to be formed for any one of them, but only around the group as a whole. In such groups there occur archegonia in which, after the division of the primary neck cell and the central cell, the latter divides several times like any other gametophyte cell. These archegonia are never as advanced as those at the micropylar end. Around some archegonia the jacket cells at the base have enlarged, resembling archegonial initials similar to the "sheath buds" described by Miss FERGUSON (11) in *Pinus*.

ARCHEGONIAL CHAMBER.—In all the material sectioned, except possibly in one group of slides, it seems that no archegonial chamber is formed. In examining some whole ovules, however, fixed about March, it would seem that the gametophytes previously sectioned had not been old enough, and that there is an archegonial chamber formed in the micropylar end by the continued growth of the gametophyte cells around the archegonia, but it is not a deep and definite chamber as is found in other cycads. The indication in the lateral groups of archegonia of an archegonial chamber is very slight.

Discussion

CALDWELL (1) considered *Microcycas* the most primitive genus of cycads. On the other hand, LAND (12) and CHAMBERLAIN (7, 8) conclude that it is an advanced form. In habit of growth it is a tree (1), like all but *Zamia*, *Stangeria*, *Bowenia*, some species of *Macrozamia*, and *Encephalartos*. The three first named are generally accepted as the most advanced cycads; therefore, in vegetative form *Microcycas* belongs to the lower cycads. In the seedling, it is to be noticed that the vascular cylinder, instead of developing from a protostelic cotyledonary plate, is siphonostelic from the beginning (10), an advanced character.

There is a tendency among cycads to reduce the microspore production by reducing the size of the sporophyll, by decreasing the number of sporangia on the sporophyll, and by diminishing the output of each sporangium. In all these respects *Microcycas* (15) is near the top of the series, with only *Bowenia* and possibly *Zamia* more advanced.

As is consistent with the tree habit, *Microcycas* has a large ovulate cone (1), but not as large as in some species of *Encephalartos*, *Macrozamia*, and *Dioon*. The cone itself ranks high in the scale of evolution, the sporophylls being very unleaflike and reduced in size. The size of ovules puts *Microcycas* among the more advanced cycads. They are smaller than those of *Cycas*, *Encephalartos*, *Macrozamia*, and *Dioon*, yet larger than those of *Zamia*, *Bowenia*, and *Stangeria*. They are about the same size as those of *Ceratozamia*, although possibly averaging larger.

The female gametophyte is quite similar to the female gametophytes of the other cycads, except in the thickness of the megaspore membrane, and in the number of the archegonia. Comparing the measurements of the megaspore membranes made by THOMSON (16) on five genera of cycads other than *Microcycas*, it would appear that *Microcycas* possesses the thinnest one to start with, and that at its greatest thickness it is approximately the same as that surrounding the free nuclear gametophyte of *Zamia integrifolia* (16), which genus is considered the most advanced of all.

Although the normal appearance of the archegonia is about like that of other cycads, their number is strikingly greater, being sixty-four or more, as compared with the one to ten archegonia found in the other genera. CALDWELL states that the number sometimes exceeds two hundred, but this number I believe to be unusual, even counting the non-functioning archegonia. Even the least number, however, does not nearly approach the narrow range of the other cycads. This situation might be called primitive. It may be that more cells retain the ability to become archegonia, given the proper stimulus. On the other hand, in view of the situation in *Welwitschia*, this large number might be regarded as an advanced character.

The development of the archegonium is similar to that of *Dioon edule*, described by CHAMBERLAIN (4), with these differences: the archegonial jacket is exhausted two or three months earlier; and a definite ventral canal cell is occasionally formed as in the Ginkgoales (3) and the Abietineae, a more primitive condition than that found in the other cycads, where there is no trace of a wall between the ventral canal nucleus and the nucleus of the egg. The archegonia occur in groups, as in the Cupressineae (13), but have no common archegonial jacket.

Another and strikingly primitive feature of *Microcycas* is the fact that there are sixteen to twenty sperms (1).

Microcycas therefore shows both primitive and advanced characters. It is to be noticed that most of the primitive characters appear in the gametophyte generation, while most of the advanced characters are in the sporophyte generation.

On the whole, *Microcycas* shows more advanced than primitive characters, and should be regarded as one of the more advanced cycads.

Summary

1. The ovules were not young enough to show the megaspore mother cell or the megaspores, the youngest stage of the female gametophyte showing free nuclear condition in the megaspore.
2. In older ovules, nuclear division has increased the number of free nuclei, while at the periphery some of the nuclei are already inclosed by walls. Still older ovules show the gametophyte to be completely cellular.
3. The number of chromosomes is twelve, although occasional nuclei have fourteen.

4. The megaspore membrane increases in thickness from 1.5 μ in August to 3 μ in October, and thereafter decreases to 1.0 μ in March.

5. The cells of the nucellus adjacent to the gametophyte are early organized into a nutritive jacket, which persists until the middle of December.

6. The vascular bundles belonging to the inner fleshy layer of the ovule divide and give rise to branches which enter the nucellus.

7. The archegonial initials are distinguishable about the latter part of November. They are scattered in groups over the surface of the gametophyte, but only those at the micropylar end develop. The remaining initials may divide and become almost unrecognizable among the other gametophyte cells, or may become merely multinucleate.

8. The development of the archegonia is the same as in other cycads, with the exception that in some cases a ventral canal cell is formed.

9. Haustoria from the egg protrude through pores in the egg membrane into the cells of the archegonial jacket which surrounds each archegonium.

10. The archegonial chamber is a shallow depression occurring late in the development of the gametophyte, and is not nearly so highly developed as in the other genera.

11. *Microcycas* should be regarded as one of the more advanced cycads.

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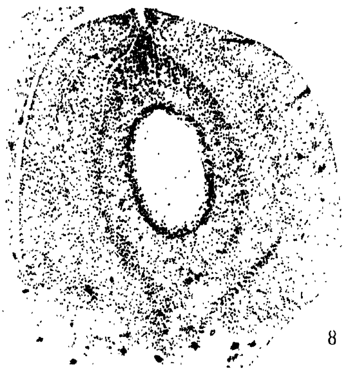
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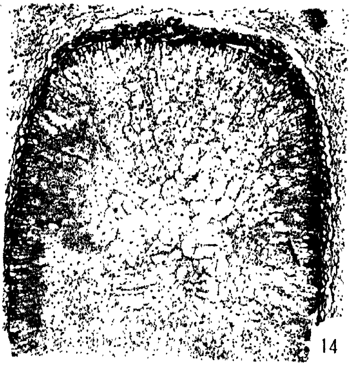
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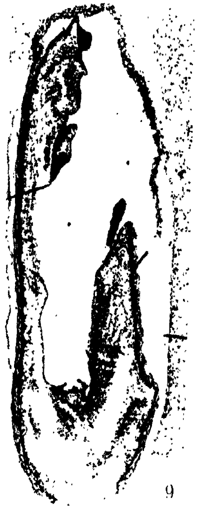
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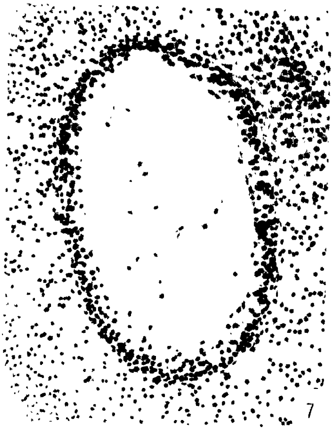
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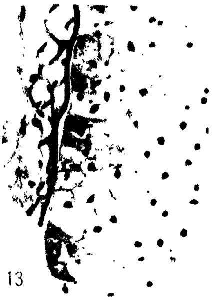
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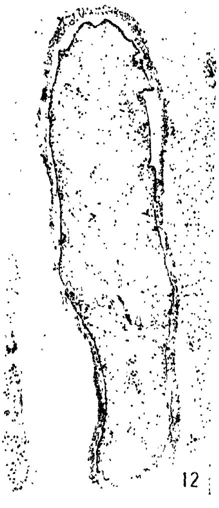
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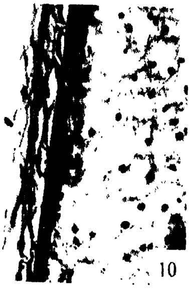
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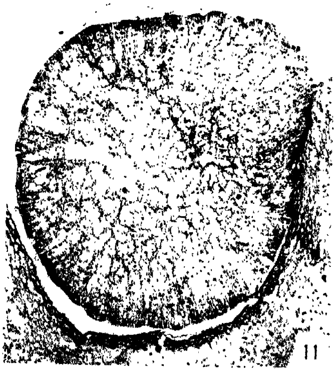
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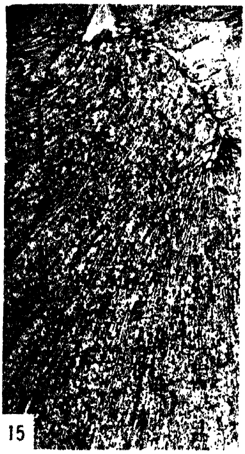
REYNOLDS on MICROCYCAS



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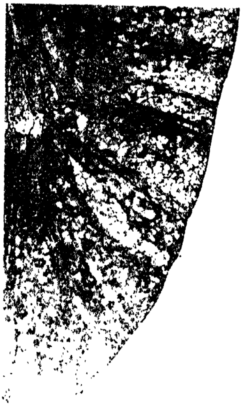

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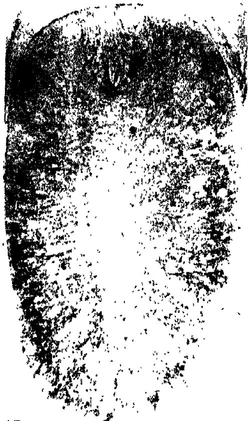

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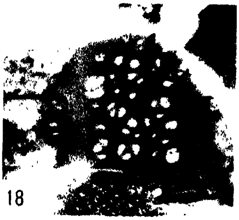

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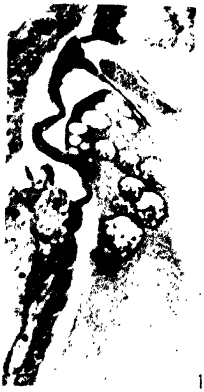

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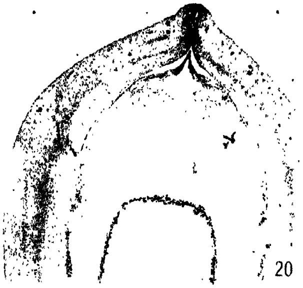

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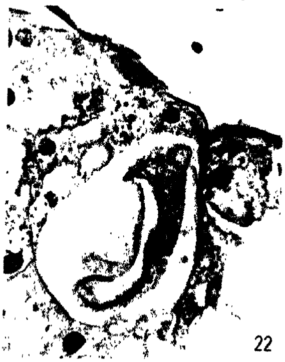

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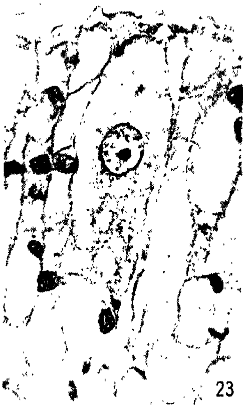

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EXPLANATION OF PLATES XXVIII, XXIX

Fig. 7.—Free nuclear stage of gametophyte with young endosperm jacket; ×67.

Fig. 8.—Young ovule containing free nuclear gametophytes surrounded by endosperm jacket; ×26.

Fig. 9.—Young, completely cellular gametophyte; ×26.

Fig. 10.—Detail of fig. 9, showing endosperm jacket, megaspore membrane, and arrangement of cells of gametophyte in regular rows; ×132.

Fig. 11.—Transverse section of young gametophyte; ×25.

Fig. 12.—Two gametophytes each within its own megaspore membrane, but inclosed by common endosperm jacket; gametophytes same age as one shown in fig. 9; ×26.

Fig. 13.—Detail of young gametophyte; note heavy walled endosperm jacket cells and regular rows of gametophyte cells; ×64.

Fig. 14.—Older gametophyte, showing location of possible archegonial initials; section not perfectly median; ×25.

Fig. 15.—Median section of mature gametophyte, showing cells in rows radiating from suture; ×16.

Fig. 16.—Tangential cut of lower part of mature gametophyte, showing alternation of light areas and dark bands; ×10.

Fig. 17.—General topography of early December gametophyte; ×6.

Fig. 18.—Detail of egg membrane in surface view; note arrangement of pores; ×190.

Fig. 19.—Multinucleate archegonium from February gametophyte; nuclei arisen from division of central cell nucleus before division to form ventral canal nucleus; ×102.

Fig. 20.—General topography of November ovule, showing partial exhaustion of nucellus; ×9.

Fig. 21.—Binucleate central cell resulting from division shown in fig. 22; note also two tiers of neck cells; archegonia of figs 21–23 are from gametophyte of age shown in fig 17; ×308.

Fig. 22.—Young archegonium in which central cell nucleus is divided; ×330.

Fig. 23.—Young archegonium in which nucleus of central cell has assumed a central position; ×308.

QUANTITATIVE RELATIONS OF CARBOHYDRATES TO NITROGEN IN DETERMINING GROWTH RESPONSES IN TOMATO CUTTINGS

MARY E. REID

(WITH EIGHT FIGURES)

The investigations described and results presented in this paper are the outcome of a study of the utilization of the different kinds of food reserves in tomato cuttings in their relation to the character of the growth responses which they display. Variations in external conditions have been used, such as growing the cuttings in light and in darkness, and in solutions with and without nitrate nitrogen.

This problem was suggested for investigation as a result of certain observations of the growth responses of tomato cuttings described by KRAUS and KRAYBILL in their work on vegetation and reproduction with special reference to the tomato.[1]

Pieces of stems one to four inches long, without leaves and possessing both nodes and internodes, were examined microchemically to learn something of the nature of their content. They were then placed on filter paper moistened with distilled water and placed under a bell jar in the laboratory. These trials were repeated several times, always with the same results. (1) Yellowish stems high in carbohydrates and low in total nitrogen and nitrates pushed forth many roots, particularly along the internodes, to the length of one to four inches. One or two formed tiny yellowish sprouts at the nodes. In ten days to two weeks the roots turned dark and began to decay. (2) Greenish stems containing starch and fairly high in total nitrogen always produced roots along the internodes and sometimes small green sprouts at the nodes. The root production was not so profuse as in the foregoing. Decay began in about the same length of time. The succulent tops of the same plants without starch reserves all decayed without root or shoot production. (3) Green, succulent stems, without starch reserves and very low in free reducing substances but high in total nitrogen and nitrate nitrogen, all decayed without root or shoot production. These results are of interest in connection with the vegetative propagation of many plants, for which purpose the practical grower prefers the more "hardened" or mature portions. From the general viewpoint expressed in this paper they are also interesting in connection with some other experiments on tomatoes which will not be discussed here, except to state that a decided reduction in the development of the root systems of the plants accompanied a continued removal of leaves from the tops. According to microchemical tests, that practice also resulted in a marked decrease in the carbohydrates in the stems, and a reduction in vegetative extension and fruitfulness.

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[1] KRAUS, E. J., and KRAYBILL, H. R., Vegetation and reproduction with special reference to the tomato. Oregon Agric. Exp. Sta. Bull. 149. 1918.

Only a brief statement will be given in this paper of the results of the writer's investigations, which were begun in the fall of 1920 and which have been continued up to the present time. It is hoped that soon a more complete account of the work may be published, giving numerical data and a survey of the literature on the subject.

The methods employed in conducting these experiments were the same as those described in a previous article.[2] The nutrient solutions were prepared according to the following formulas:

Solution containing nitrates

A	B
2 per cent $MgSO_4$	3 per cent $CaCl_2$
2 per cent $KH_2\,PO_4$	2 per cent $CaSO_4$
2 per cent KNo_3	4 per cent $Ca\,(NO_3)_2$

Solution minus nitrates

A	B
2 per cent $Mg\,SO_4$	4 per cent $CaCl_2$
2 per cent $KH_2\,PO_4$	2 per cent $CaSO_4$
1 per cent KCl	

In preparing the solutions for the cuttings, 100 cc. of solution A was made up to one liter in distilled water, and an equal quantity of solution B was made up in the same way. These diluted solutions of A and B were mixed before using. A few drops of a 1 per cent solution of ferric citrate were added to each liter of the solution.

Observations

In the previous paper the text figures were so arranged as to show the effect of differences in composition upon the growth of cuttings under uniform external conditions. In this article the cuttings of uniform composition are shown on one text figure, but a comparison of the effects of differences in composition may be seen by referring to the figures on opposite pages. For example, figs. 1 and 3 are of basal cuttings of the two types of composition. To obtain an idea of the behavior of cuttings dissimilar in composition but grown under the same external conditions, compare cuttings *A* and *A* in the two figures, *B* and *B*, etc.

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[2] Reid, Mary E., Relation of the kind of food reserves to regeneration in tomato plants. Bot. Gaz. 77: 103–110. 1924.

Fig. 1 shows basal carbohydrate-high cuttings grown under different external conditions. Cutting *A* was grown in the light in the solution plus nitrates, *B* was grown in the dark in the solution plus nitrates, *C* was grown in the light in the solution minus nitrates, and *D* was grown in the dark in the solution minus nitrates. It may be observed that roots grow abundantly on cuttings kept in light and in darkness in both plus nitrate and minus nitrate solutions. There are striking differences in shoot growth under the different external conditions. There are more shoots and a greater weight of shoots produced in light than in darkness in solutions both plus and minus nitrates, but perhaps the most conspicuous feature is that shoots grow only to a small extent and often not at all on cuttings kept in the solution minus nitrates.

In another series of experiments the cuttings were obtained from plants that had been transplanted into sand at an earlier age than the plants from which the cuttings here shown were taken. The nitrogen content of these plants was presumably less than that of the plants transplanted at the earlier age. The growth responses made by these cuttings is shown in fig. 5 (p. 412). This cutting was high in carbohydrates and was grown in the light in the solution minus nitrates. No shoots were produced on any of the basal cuttings in this series which were grown in the solution minus nitrates, but roots were produced abundantly.

These growth responses of cuttings high in carbohydrates and low in nitrogen indicate that nitrate nitrogen may be utilized in darkness in the building of nitrogenous materials that may be used in growth, but the production of such materials in darkness is much less than occurs in light. The addition of nitrates to the nutrient solution often resulted in twice the amount of growth that occurred on cuttings grown in the solution minus nitrates. The difference in the amount of growth in the two solutions is largely a matter of increased shoot production by the cuttings grown in the solution plus nitrates.

The results obtained with high carbohydrate, low nitrogen cuttings grown in the solution minus nitrates appear to indicate that the nitrogenous reserves in defoliated cuttings are utilized more fully when the cuttings are grown in light than when they are grown in darkness. This difference is largely a matter of difference in shoot growth. It seems that cuttings grown in the light are much more likely to produce shoots.

Fig. 2 shows basal cuttings of highly vegetative plants. Cuttings *A* and *B* were grown in the solution plus nitrates, and *C* and *D* in the solution minus nitrates. *A* and *C* were grown in the light, and *B* and *D* in darkness. It may be observed that vegetative cuttings grown in the light produce some shoots, but do not produce roots abundantly. In most cases none is produced until after the cuttings have grown in the light for several weeks.

Fig. 3 shows top cuttings of carbohydrate-high plants. *A* and *B* were grown in the solution plus nitrates, and *C* and *D* in the solution minus nitrates. *A* and *C* were grown in the light, and *B* and *D* in darkness. It may be noted that the cuttings grown in the light in the solution plus nitrates developed both roots and shoots abundantly. In darkness, both roots and shoots were very limited in quantity. When grown in the solution minus nitrates, the top cuttings produced, at the end of a growing period of about one month, a relatively greater weight of shoots than did the basal cuttings, but relatively less roots.

Fig. 4 shows top vegetative cuttings. *A* and *B* were grown in the solution plus nitrates, and *C* and *D* in the solution minus nitrates. *A* and *C* were grown in the light, and *B* and *D* in darkness. The photograph was taken after the cuttings had been growing in their respective solutions for two weeks. Shoots were produced by cuttings grown in the light about equally well in the two solutions. No roots were developed. Neither roots nor shoots grew in darkness, but a withering of the tips was observable after the cuttings had stood in the dark room for five to seven days.

The results obtained with vegetative cuttings in all the experiments thus far conducted have indicated that the vegetative cuttings having the highest carbohydrate supply are the ones that produce the most new growth in darkness. Those cuttings containing

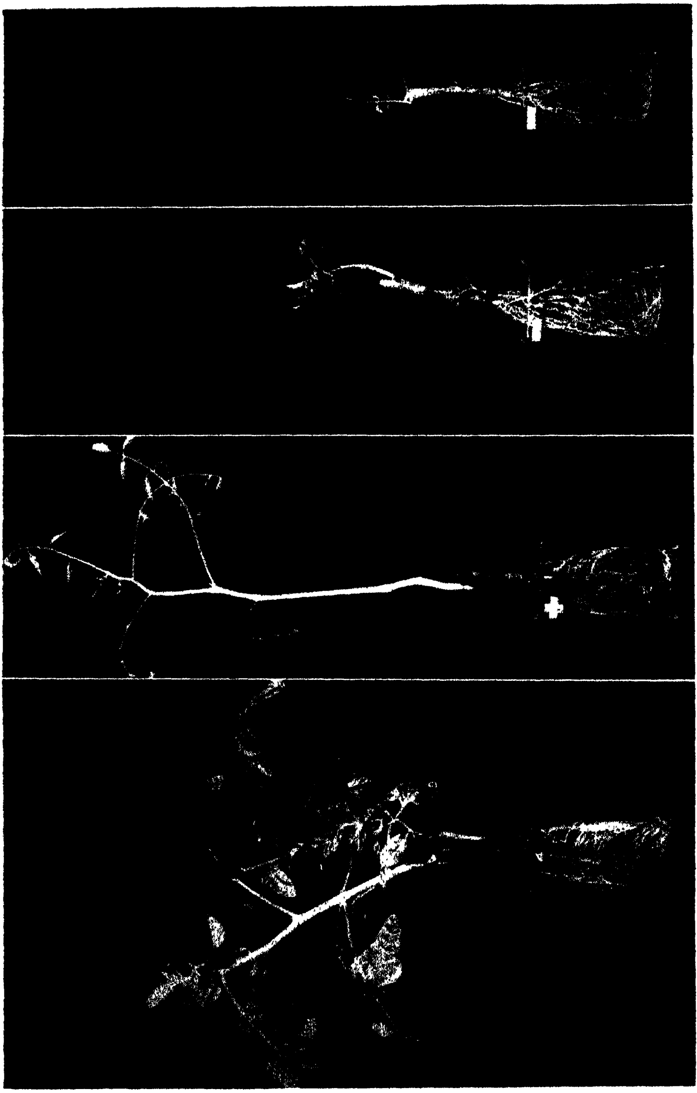

FIG. 1.—Basal cuttings of carbohydrate-high plants; cuttings grown in solution plus nitrates: *A*, in light; *B*, in darkness; cuttings grown in solution minus nitrates: *C*, in light; *D*, in darkness.

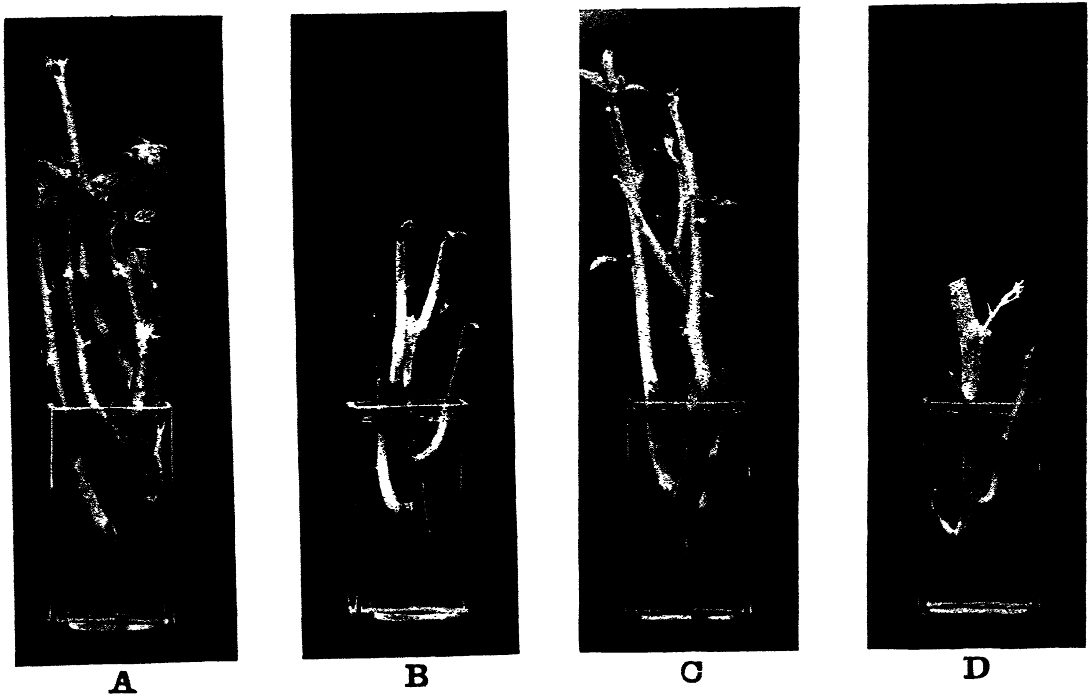

FIG. 2.—Basal cuttings of vegetative plants; cuttings grown in solution plus nitrates: *A*, in light; *B*, in darkness; cuttings grown in solution minus nitrates: *C*, in light; *D*, in darkness.

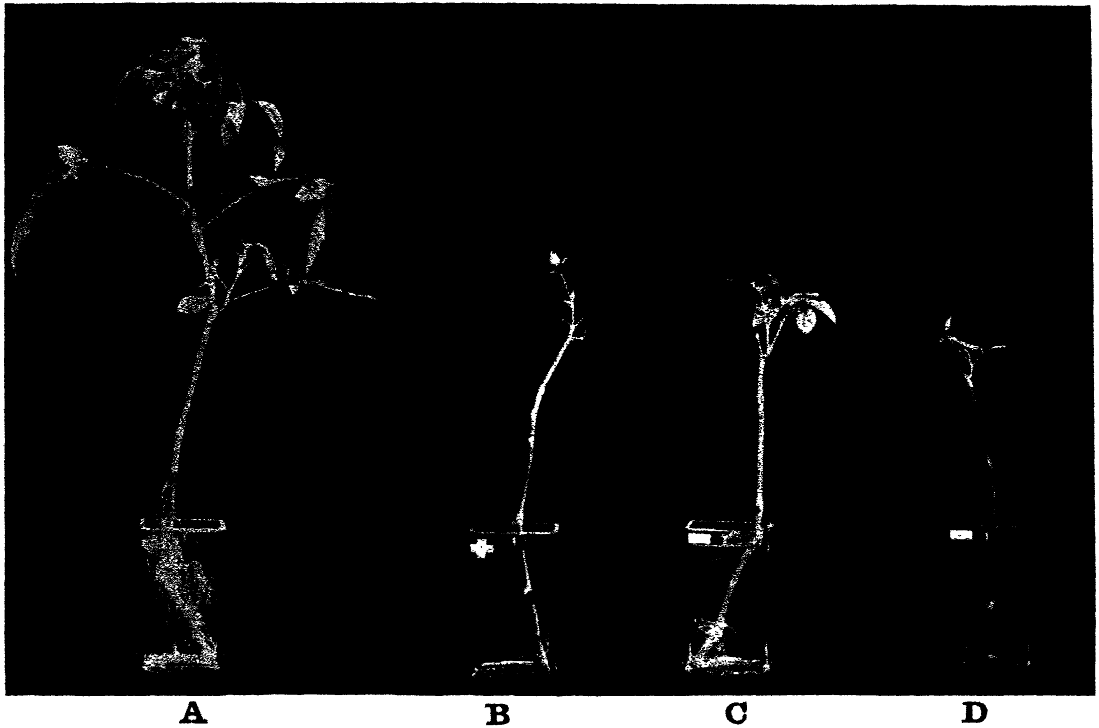

FIG. 3.—Top cuttings of carbohydrate-high plants; cuttings grown in solution plus nitrates: *A*, in light; *B*, in darkness; cuttings grown in solution minus nitrates: *C*, in light; *D*, in darkness.

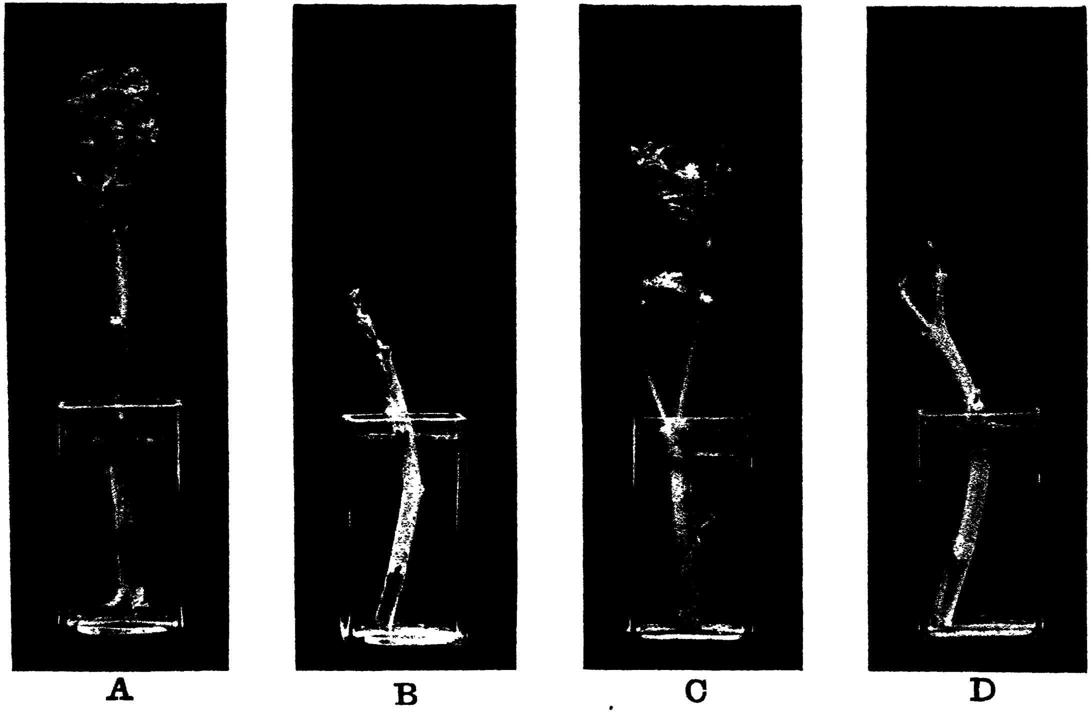

FIG. 4.—Top cuttings of vegetative plants; cuttings grown in solution plus nitrates: *A*, in light; *B*, in darkness; cuttings grown in solution minus nitrates: *C*, in light; *D*, in darkness.

the greatest amount of young tissue, such as the top cuttings or the basal cuttings with long side shoots, produce the greatest total amount of new shoots when grown in the light. During the first two weeks of the growing period of highly vegetative cuttings, shoots appear to develop equally well in the solutions plus nitrates and minus nitrates. This was true both in light and in darkness. Later, however, those cuttings growing in the light in the solution plus nitrates had the greatest total amount of new shoots.

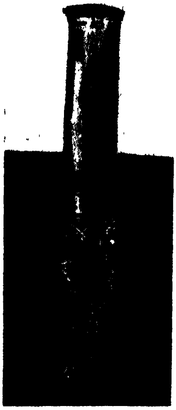

FIG. 5.—Basal cutting of carbohydrate-high plant presumably having lower nitrogen content than cutting shown in fig. 1 *C* (both grown in light in solution lacking nitrates).

Fig. 6 shows top cuttings of vegetative plants grown in the light and grown a week longer than similar cuttings shown in the last figure, in which no roots were present. Cuttings *A–D* inclusive were grown in the solution minus nitrates and *E–H* inclusive in the solution plus nitrates. It has been found in most of the experiments with top vegetative cuttings that, in the first two weeks of the growing period, roots develop in greatest abundance upon cuttings grown in the solution minus nitrates, either in light or in darkness. Later, in the case of cuttings grown in the light in solutions both plus and minus nitrates, the roots appear in equal amounts. Still later, the roots appear to develop more rapidly on the cuttings growing in the solution plus nitrates. This difference between earlier and later periods of growth is believed to be correlated with the larger photosynthesizing area which the cuttings grown in the solution plus nitrates eventually develop. The growth responses appear to indicate that, if the carbohydrate supply becomes the limiting factor, the growth of shoots exceeds that of roots; and that if, on the contrary, the nitrogen supply is the limiting factor, the growth of roots may exceed that of shoots. Considering the growth responses of the highly vegetative cuttings just described, it seems that a large reserve supply of carbohydrates is not directly essential to root growth. Apparently only as the materials enter into solution

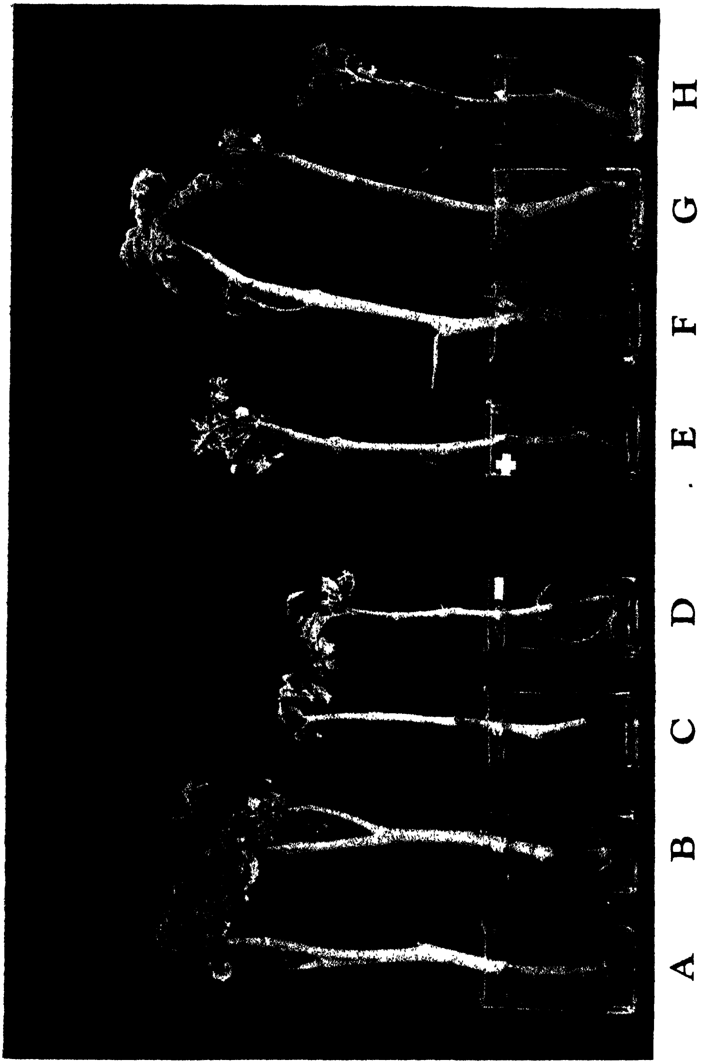

FIG. 6.—Top cuttings of vegetative plants grown in light: *A–D*, in solution lacking nitrates; *E–H*, in solution containing nitrates.

do they exert an influence upon the type of growth. At the time these highly vegetative cuttings were made, they contained practically no starch except a few grains in the endodermal cells. After the cuttings developed leaves, carbohydrates were eventually synthesized faster than they were utilized, and there occurred a deposition of starch in typical starch storage cells. An accumulation of carbohydrates occurred earlier in the cuttings grown in the solution minus nitrates than in those grown in the solution plus nitrates. It is not thought that starch itself is the determining factor, but rather that some other conditions accompanying starch deposition may be important, as, for example, a high sugar content.

There appears to be a close relationship between the type of growth response displayed by cuttings and that of the plant from which the cuttings are obtained. The cuttings seem to have a tendency to do what the plant has been doing. If the plants were producing shoots to a small extent but producing roots abundantly, the basal cuttings of such plants tend to produce chiefly roots, and the top cuttings to produce both roots and shoots, the combined effect being a greater total tendency toward root production. If the plant were producing shoots abundantly but roots to a small extent, the basal cuttings produce shoots and a few roots, the tops only shoots, the combined effect being a greater total tendency toward shoot production.

In fig. 7, plant *A* is highly vegetative and of the series of plants from which the vegetative cuttings shown in figs. 2, 4, and 6 were taken. Branches have been produced at most of the nodes, and the leaves are exceedingly large, dark green, and numerous. The root system consists of relatively few, thick, fibrous, short, slightly branched roots. Plant *D* is the carbohydrate-high type from the same series. Its stem is straight and unbranched. The leaves are few, small, and a very light yellowish green. On the other hand, the root system is relatively very large. The roots are very numerous, and, although not long, possess a large surface area in relation to the small size of the plant above ground. The plant shown in illustrations *B* and *C* is not strictly comparable, since it is slightly older than plants *A* and *D*. It is interesting to note that this plant is intermediate in chemical composition, as well as in the relative amounts of roots and shoots.

In fig. 8 are shown iodine-treated sections taken through the basal portion of the stems from the three plants shown in fig. 7. *A* and *B* are sections through the basal portion of the carbohydrate-high plant. The darkened areas in *B* show the heavy deposit of starch in the pith and medullary ray cells. *A* is a section through the same stem, taken through a region in which the starch was not so abundant as in *B*, and in which the individual grains show plainly. Section *D* is through the basal portion of the highly vegetative plant, no starch grains being visible. The tissues shown are the older portion of the xylem in the region of one of the primary vascular bundles, pith cells, and a group of internal phloem cells. Section *C* is through the stem intermediate in type. Considerable starch was present, but very much less than in the carbohydrate-high type of plant.

Discussion

The results of these experiments seem to indicate that the growth responses as expressed in root and shoot production may be determined, at least in part, by the nature of the available food materials. It seems that, if the carbohydrate supply becomes the limiting factor, the growth of tops exceeds that of roots, but that if the nitrogen supply becomes the limiting factor, the growth of roots may exceed that of tops. It seems probable that the relationship must be indirectly between the relative amounts of certain forms of soluble carbohydrates present and the supply of some inorganic form of nitrogen. As a result of the relative proportions of these constituents, organic nitrogen compounds are formed which directly determine the nature of the growth response. It is supposed that a supply of carbohydrates must be present in excess of that used in forming these nitrogenous organic compounds, in order to provide materials for respiration and for building cell walls. It might be assumed as a result of these experiments that merely a difference in the total amount of the organic nitrogen compounds would determine differences in root and shoot growth. Experiments now under way, however, suggest that total nitrogen or total protein content may not explain differences in relative amounts of roots and shoots produced. These same experiments suggest that, given a readily available supply of carbohydrates, it is the balance

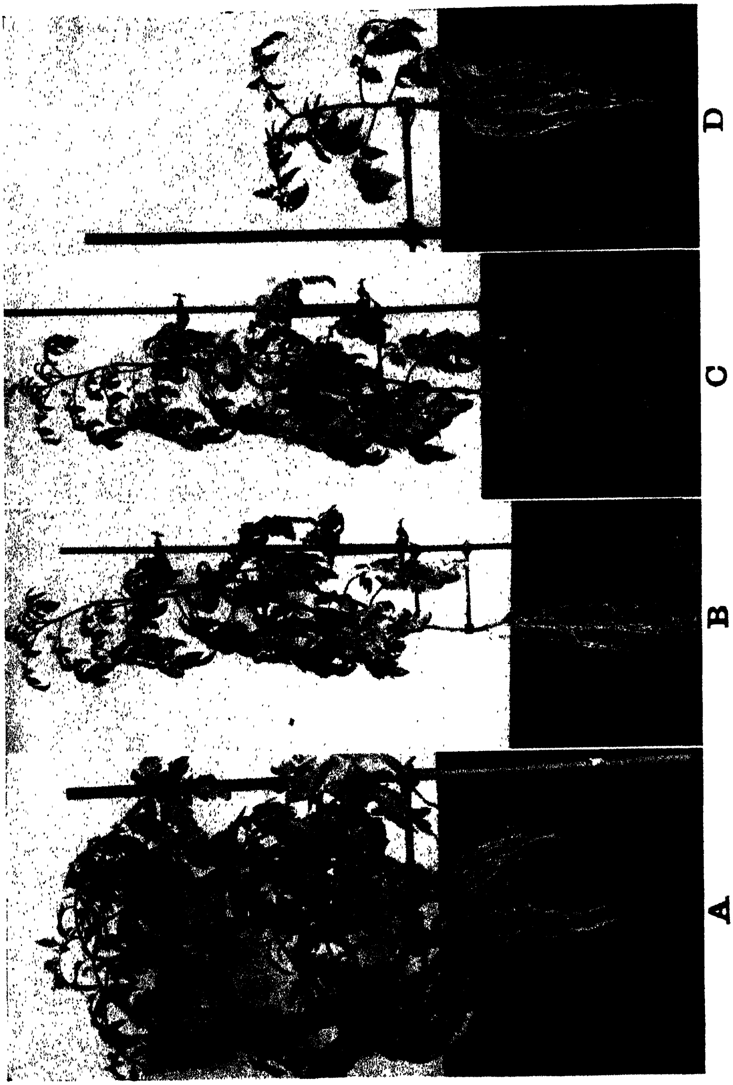

FIG. 7.—*A*, highly vegetative plant; *B*, *C*, plant moderately vegetative; *D*, carbohydrate-high plant

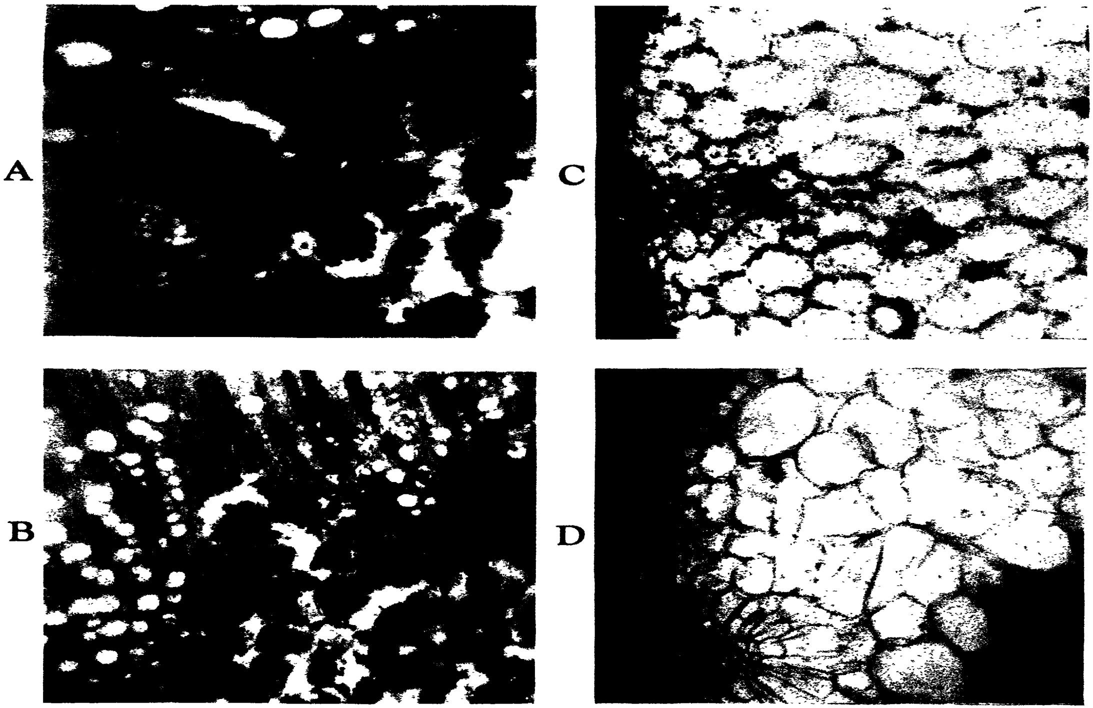

Fig. 8.—Microphotographs of iodine-treated sections taken through basal portions of stems of plants shown in fig. 7, illustrating differences in relative amounts of starch stored: *A*, *B*, sections through stem of carbohydrate-high plant illustrated in fig. 7 *D*; *C*, section through stem of moderately vegetative type of plant shown in fig. 7 *B*, *C*; *D*, section through stem of highly vegetative plant shown in fig. 7 *A*.

that exists between some forms of organic nitrogen that determines the growth responses.

Summary

1. A high nitrogen supply plus a readily available (even though somewhat limited) supply of carbohydrates appears to furnish favorable conditions for shoot growth.

2. A somewhat limited nitrogen supply plus a readily available supply of carbohydrates appears to furnish favorable conditions for root growth.

3. There seems to be a greater utilization of the carbohydrate reserves in carbohydrate-high cuttings grown in light than in those grown in darkness, and the utilization is greater in the cuttings grown in the solution plus nitrates than in those grown in the solution minus nitrates.

4. A synthesis of nitrogenous materials that may be used in growth appears to occur in cuttings grown both in light and in darkness, but such synthesis appears to be greater in amount in light than in darkness.

5. Light is particularly favorable for the growth of shoots.

6. The cuttings have a tendency to do what the plant has been doing. If the plant was producing shoots abundantly and roots to a relatively small extent, the basal cutting from such a plant tends to produce a few roots and some shoots, and the top cutting chiefly shoots, the combined effect being a greater total tendency toward shoot production. If the plant was producing roots abundantly and shoots to a small extent, the basal cutting tends to produce only roots, the top cutting to produce roots and some shoots; the combined effect being a greater total tendency toward root production.

These investigations were conducted in the laboratory of plant physiology of the department of botany at the University of Wisconsin, and were begun at the suggestion of Professor E. J. KRAUS. The writer wishes to extend grateful acknowledgment to Professors J. B. OVERTON and E. J. KRAUS for helpful suggestions and criticisms given during the progress of the investigations.

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EFFECT OF POTASSIUM ACID PHTHALATE ON EARLY GROWTH OF TOMATO PLANTS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 319

R. B. Dustman

(WITH SIX FIGURES)

In connection with a study of the effect of reaction values of nutrient cultures upon certain internal factors in plant metabolism, it became desirable to know the effect, if any, of the organic compound, potassium acid phthalate, upon the early growth and vigor of plants subjected to its action. So far as the writer is aware, the use of phthalic acid or its salts in nutrient cultures has been reported from a single source, the work of Tarr and Noble (6). These investigators employed potassium acid phthalate for the purpose of stabilizing the hydrogen ion concentrations of their nutrient solutions. They report excellent buffer action from its use, but state that they were unable to determine definitely whether or not it exerted a harmful effect upon the plants grown. The results were regarded as being indicative of no such effect. It is known, however, that many of the benzene ring derivatives may exert a toxic influence upon growth. Thus Brenchley (1) found an inhibitory action from various phenols, all of which produced the same general effect, but which varied considerably in respect to the concentrations at which these effects became apparent. Schreiner and Skinner (5) showed that relatively small amounts of salicylic aldehyde were toxic to the growth of various plants in water cultures and in pot and field experiments. Unpublished data from this laboratory indicate that benzidine is quite harmful to the growth of soy beans and barley. Unfortunately none of these compounds is sufficiently closely related to the one in question to permit comparison. For these reasons five series of experiments were arranged and carried out in a small but carefully conducted manner.

Series I.—Sand cultures in half gallon jars; five lots including 000, 1000, 2000, 4000, and 10,000 ppm of phthalate; T and N nutrient solution; P_H range 5.03–5.05; three plants per jar; variety Bonny Best.

SERIES II.—Sand and water cultures in glass stenders; six lots of each including 000, 500, 1000, 1500, 2000, and 2500 ppm of phthalate; T and N nutrient solution; P_H range 5.02–5.07; three plants per stender; variety Bonny Best.

SERIES III.—Sand cultures in half gallon jars; six lots including 000, 500, 1000, 1500, 2000, and 2500 ppm of phthalate; NRC Type III R_6S_1, nutrient solution; P_H range 3.73–3.83; three plants per jar; variety Bonny Best.

SERIES IV.—Sand cultures in glass stenders; six lots each of barley and tomatoes including 000, 200, 400, 600, 800, and 1000 ppm of phthalate; T and N nutrient solution; P_H range 5.02–5.06; four plants per stender; varieties Wisconsin pedigreed barley and Stone tomatoes.

SERIES V.—Soil cultures in half gallon jars; six lots including 000, 125, 250, 500, 750, and 1000 parts of phthalate ppm of soil; five plants per jar; variety Stone.

Methods

All experiments were run in duplicate. The plants were grown under greenhouse conditions and consisted, in all instances but one, of tomatoes of the Bonny Best and Stone varieties. The single exception was a parallel set of pure line barley cultures grown as a check against the tomatoes in the series containing the lowest concentrations of phthalate. Water, sand, and soil cultures were employed, but the former proved unsatisfactory. Most of the cultures were of sand media arranged partly in large glass stenders of 250 cc. capacity, and partly in half gallon jars of glazed earthenware. The glass containers were wrapped with black glazed paper to protect the roots from the action of light. In all, ranges of 200–10,000 ppm of phthalate were tried.

Two types of nutrient solutions were employed, including the one used by TARR and NOBLE (6) and the National Research Council (4) type III, row 6, solution 1. The latter was chosen because of its reported hydrogen ion concentration, which according to MCCALL and HAAG (3) is P_H 3.9 by the colorimetric method, but which as made up and measured in these experiments gave a reaction value of P_H 3.73 with the Leeds and Northrup Type K potentiometer, equipped with a modified HILDEBRAND (2) type of hydrogen electrode and a saturated calomel electrode with liquid junctions across saturated KCl solution. This instrument was used in adjusting the various nutrient solutions of the sand and water cultures to a uniform value of approximately 5 P_H by the addition of tenth normal sodium hydroxide. The actual range of all the nutrient cultures in their initial reaction values was P_H 5.02–5.07, except the National Research Council type III solutions, which were taken at their resulting values of P_H 3.73–3.83 after addition of the varying increments of phthalate and without adjustment with sodium hydroxide.

The sand used was from a fresh supply of pure white quartz of high quality and medium texture (no. 3 grade) prepared by the Wausau Abrasive Company, Wausau, Wisconsin. It was used directly without washing or other previous treatment. The chemicals were of standard purity as supplied by the Baker Chemical Company and the Central Scientific Company of Philadelphia and Chicago, respectively. The soil used was a rich potting soil from the greenhouse, heavily manured but otherwise unfertilized. Its reaction value was undetermined, but could not well have been very acid, as it contained numerous small shells of carbonate of lime. In preparing the pots the dry soil was spread out and the phthalate applied in solution. After thorough mixing the soil was returned to the jars.

In the series containing the water cultures these were equipped with paraffin mounts with small holes through which the previously germinated seedlings were thrust into the solution beneath.

No applications of either nutrient materials or potassium acid phthalate were made subsequent to the initial application in any of the experiments, and no attempt was made to control reaction values after the seeds were planted. Waterings were made with distilled water alone, the stender cultures receiving equal volumes until differences in growth necessitated adjustment in accordance with transpiration, whereas the pot cultures were held at uniform moisture content by frequent, usually daily, weighings. Growth periods ranged from twenty to thirty-five days, except for the water cultures, which were discontinued after two weeks.

Data

Series I was set up and planted March 8 with ten seeds per pot, at which time was applied also 500 cc. of the T and N adjusted nutrient solution. The plants were later thinned to three per jar. Table I shows the results secured.

Series II (including both sand and water cultures) was started April 10 and 11. The water cultures were supplied with 200 cc. of adjusted T and N solution and three seedlings each. By the end of the second week it was apparent that these cultures were unreliable. They therefore were discontinued. Several of the solutions were showing contamination with mold and algal growth, and there was some indication that the paraffin injured the seed leaves where they rested upon it, the sunlight and temperature being sufficient to soften and slightly melt the surface of the mounts. The sand cultures of this series proved to be much more satisfactory. They were treated with 90 cc. of T and N adjusted nutrient solution, planted with five sprouting seeds each, and four days later thinned to three seedlings each. The results are shown in table II.

TABLE I

EFFECT OF STRONG CONCENTRATIONS OF KH PHTHALATE ON GROWTH OF TOMATO PLANTS (GROWTH PERIOD 35 DAYS)

Lot no.	KH phthalate ppm	N/10 NaOH added per liter (cc.)	Initial P_H	Final dry weight per plant (gm.)	Final P_H
1..........	None	86	5.05	0.0759	3.85
2..........	1000	110	5.04	0.0707	6.64
3..........	2000	135	5.04	0.0174	7.46
4..........	4000	180	5.03	Dead	....
5..........	10,000	330	5.05	Dead	....

Series III contained the same concentrations of phthalate as Series II, but was based upon a totally different nutrient solution. As already stated, the National Research Council type III R_6S_1 was chosen because of its reported reaction value of P_H 3.9. As made up and used with the addition of phthalate and without adjustment with NaOH, the initial range of reaction values was

P_H 3.73–3.83. The half gallon jars of sand were planted April 14 with seven previously soaked seeds in each, at which time 500 cc. of the solution was applied. These were later thinned to three plants per jar. Table III shows the yields secured.

Series IV was carried out in stenders and included parallel sets of tomatoes and barley. The solutions for both were made up together and divided between the two sets. On April 27 the cultures were set up with 100 cc. of T and N adjusted solution and planted with seven barley grains or ten tomato seeds each. When well up the plants were thinned to four per culture. The barley plants were harvested May 19 and the tomatoes May 24. The yields are shown in tables IV*a* and IV*b*.

TABLE II

EFFECT OF MODERATE CONCENTRATIONS OF KH PHTHALATE ON GROWTH OF TOMATO PLANTS (GROWTH PERIOD 20 DAYS)

LOT NO.	KH PHTHALATE PPM	N/10 NAOH ADDED PER LITER (CC.)	P_H	AVERAGE WEIGHT PER PLANT	
				Green gm	Dry gm.
1	None	86	5.06	1.0043	0.0958
2	500	100	5.07	0.8734	0.0786
3	1000	110	5.02	0.5006	0.0449
4	1500	125	5.04	0.4236	0.0392
5	2000	135	5.06	0.2858	0.0280
6	2500	145	5.05	0.2536	0.0263

TABLE III

EFFECT OF MODERATE CONCENTRATIONS OF KH PHTHALATE ON GROWTH OF TOMATO PLANTS (GROWTH PERIOD 35 DAYS)

LOT NO.	KH PHTHALATE PPM	P_H	AVERAGE WEIGHT PER PLANT	
			Green gm.	Dry gm.
1	None	3.73	4.3087	0.4317
2	500		2.7402	0.2995
3	1000		2.0531	0.1887
4	1500		1.2359	0.1082
5	2000		1.3660	0.1270
6	2500	3.83	0.3607	0.0411

Series V included the single group of soil cultures tried. The jars were planted April 28 with twelve seeds each, and a week later thinned to five plants per jar. They grew vigorously and were harvested May 24. The results are shown in table V.

TABLE IV*a*

EFFECT OF WEAKER CONCENTRATIONS OF KH PHTHALATE ON GROWTH OF TOMATO PLANTS (GROWTH PERIOD 27 DAYS)

LOT NO.	KH PHTHALATE PPM	N/10 NAOH ADDED PER LITER (CC.)	P_H	AVERAGE WEIGHT PER PLANT	
				Green gm.	Dry gm.
1..........	None	86	5.06	2.2919	0.2304
2..........	200	93	5.05	2.0042	0.2074
3..........	400	97	5.04	1.8822	0.1964
4..........	600	101	5.03	1.7427	0.1785
5..........	800	106	5.04	1.7403	0.1938
6..........	1000	110	5.02	1.5414	0.1761

TABLE IV*b*

EFFECT OF WEAKER CONCENTRATIONS OF KH PHTHALATE ON GROWTH OF BARLEY PLANTS (GROWTH PERIOD 22 DAYS)

LOT NO.	KH PHTHALATE PPM	N/10 NAOH ADDED PER LITER (CC.)	P_H	AVERAGE WEIGHT PER PLANT	
				Green gm	Dry gm.
1..........	None	86	5.06	1.0455	0.1492
2..........	200	93	5.05	0.9998	0.1274
3..........	400	97	5.04	0.7595	0.1056
4..........	600	101	5.03	0.5433	0.0758
5..........	800	106	5.04	0.7327	0.0951
6..........	1000	110	5.02	0.6473	0.0893

Discussion

The general course of development in these experiments was from stronger to weaker concentrations of phthalate. As soon as it became apparent that a given series was showing a harmful effect from the phthalate added, a new series in which the concentration was reduced was put under way. The primary object was to determine whether or not the phthalate exerted a toxic action on tomato plants, in concentrations suitable for buffering the solutions in which they were grown. Consequently its effect in concentrations below 200 ppm was not studied. TARR and NOBLE had already shown that it exerts a good buffer action at a concentration of 1020 ppm in the solution employed by them. It was thought that perhaps even greater concentrations could be used without harmful results, thus necessitating less frequent changes of solutions, and still maintain constant reaction values. The outcome of the trials herein reported not only demonstrates the fallacy of this view, but raises a serious question as to the freedom of the solutions, from toxic influences when containing potassium acid phthalate in concentrations considerably below 1000 ppm.

TABLE V

EFFECT OF KH PHTHALATE ON TOMATO PLANTS GROWN IN RICH SOIL (GROWTH PERIOD 27 DAYS)

LOT NO.	PARTS OF KH PHTHALATE PPM OF SOIL	AVERAGE WEIGHT PER PLANT (TOP ONLY)	
		Green gm.	Dry gm
1	None	16.38	1.115
2	125	17.40	1.279
3	250	18.20	1.269
4	500	18.74	1.383
5	750	18.24	1.272
6	1000	19.00	1.509

Table I shows that the seedling tomato plants were unable to endure the stronger concentrations. In this connection it should be stated that the very much increased osmotic concentration incident to the additions of phthalate and sodium hydroxide probably contributed materially to their failure. In fact, there were several variables in this work whose partial effects were difficult to separate and evaluate, especially in the higher concentrations. These were the potassium acid phthalate, sodium hydroxide, total osmotic concentration, and reaction value. The three last mentioned are believed to have been eliminated, so far as harmful effects are concerned, in the later experiments embracing the lesser concentrations of phthalate and reduced total osmotic values.

There are certain inherent difficulties involved in the use of culture solutions where concentration of hydrogen ion is an important feature in the studies being made. No data are known to the writer which might suggest the reaction value most favorable for the growth of tomato plants. The fact, however, that most plants tried have shown satisfactory growth from P_H 4 to 6, and a general knowledge of the tomato as applied to its growth under field conditions, would lead one to believe that it is not particularly sensitive to moderately acid conditions, and that these values would permit normal development. Accordingly, a P_H value of five was arbitrarily chosen for the majority of the solutions, although they probably did not remain continuously at this value.

A comparison of tables II and III indicates that the phthalate is the important factor entering into the reduction of growth, since it is active alike at P_H 3.7 and 5.00, and in solutions of dissimilar composition. This comparison also eliminates the sodium hydroxide and sodium ion from consideration at these concentrations, since the former was not added in series III, and no sodium salt entered into the composition of the solution.

From series IV it is apparent that the toxic action of the phthalate is not specific for tomatoes, as it caused a somewhat greater relative reduction with barley than with tomatoes. The yields are somewhat irregular here, as elsewhere in the experiments, but some inconsistency is to be expected where the increments of change are small. The general trend of the effect is obvious throughout. In each of the first four series the effect of the phthalate was manifest not alone by growth differences. With increasing concentration its action was reflected in the color, appearance, and general vigor of the plants. The barley plants developed a chlorotic condition roughly proportional to the amount of phthalate present in solution. Photosynthetic disturbances were more apparent with the barley than with the tomato plants. In the higher concentrations, the tomatoes showed some tendency toward malformation of leaves and a very much greater susceptibility to damping off in the seedling stage. When the roots were washed out it was found that their development was more shallow in the stronger concentration of phthalate. In general the extent of the root development was about proportional to the top growth, but even when considerably developed laterally they frequently failed to penetrate deeply into the sand.

There were several reasons for not repeating the treatments or changing the solutions during the growth period. Uncertainties as to osmotic relations, reaction values, and parallel treatments of duplicates militated against such a procedure. That changes occurred from those conditions originally present in the solutions is almost a certainty. The extent of such changes is for the most part unknown. Nevertheless, if an initial application of potassium acid phthalate results in a toxic action, it seems reasonably certain that its continued presence in the original concentration would not be less harmful. Figs. 1–6 show the plants of the various series

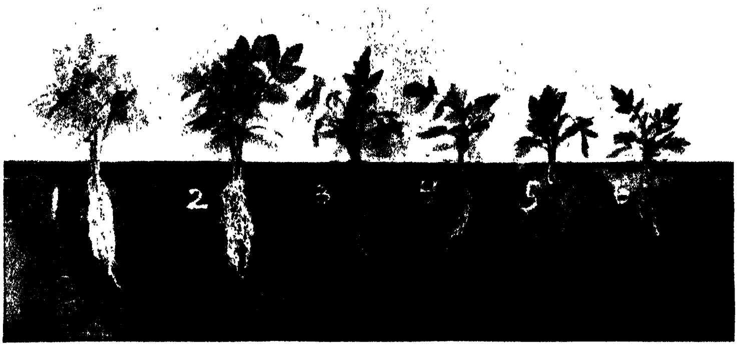

FIG. 1.—Series II: showing decreased growth with increasing phthalate treatment 20 days after planting; range 0–2500 ppm.

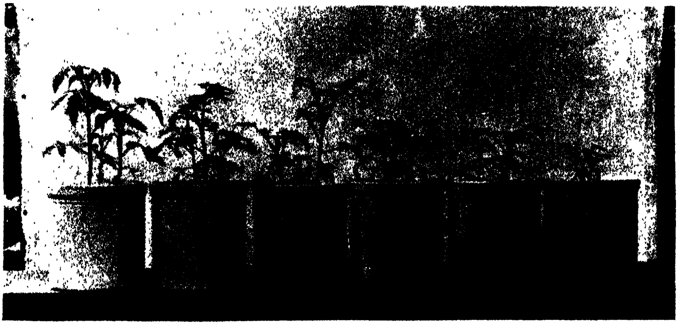

FIG. 2.—Series III: same phthalate treatment as in fig. 1, but with different nutrient solution; 35 days after planting.

at the time of harvest, and give some idea of the relative growths secured.

Series V presents an interesting reversal of the results secured from the sand and water cultures. It was anticipated that the toxic effect of the phthalate would be less pronounced in the soil cultures. That the compound might actually show a positive beneficial effect was entirely unexpected. The close agreement of the duplicates at the two extremes of the series, although not evident from the table, leaves little room for doubt as to its action. In

FIG. 3.—Series IV: effect of less concentrations of phthalate on tomatoes 27 days from planting; range 0–1000 ppm.

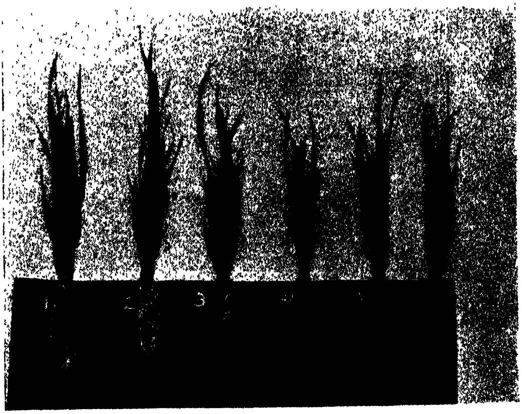

FIG. 4.—Series IV: same treatment as in fig. 3 on barley 22 days from planting

searching for an explanation for this effect, two possibilities suggest themselves: (1) the phthalate ion may have been removed from solution by adsorption; or (2) it may have been more rapidly attacked and broken down by the much more numerous mold and bacterial flora of the soil. Of the two, the former would seem to be the more likely explanation, as no differences were apparent during the first fifteen to eighteen days of growth. Presumably the potassium component of the phthalate was responsible for the increased growth. The outcome of this experiment might serve as an illustration of the dangers involved in a too general application of observed phenomena or results secured under specific conditions to a parallel but somewhat different situation.

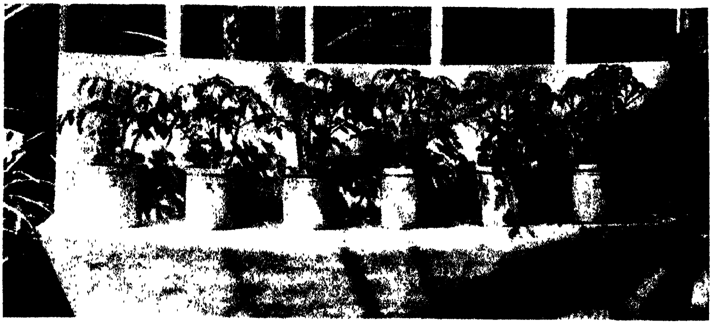

FIG. 5.—Series V: showing increased growth with increasing phthalate treatment on soil cultures 27 days after planting; range 0–1000 ppm.

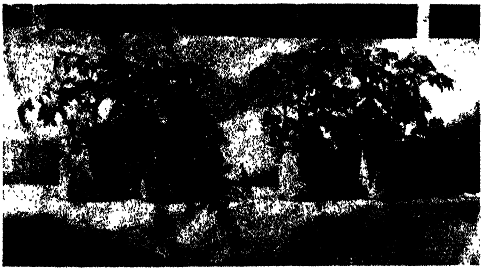

FIG. 6.—Series V: same as fig. 5, showing duplicate pots in lots 1 and 6

Summary

A study has been made of the effect of varying concentrations of potassium acid phthalate on the early growth of tomato plants. Culture experiments were carried out under greenhouse conditions, and included water, sand, and soil media. In all, ranges of 200 to 10,000 ppm of the phthalate were tried. With one exception the nutrient solutions of the sand and water cultures were adjusted to a uniform reaction value of 5 P_H by the addition of tenth-normal sodium hydroxide. In this one instance a nutrient solution of different type was employed. Its reaction value was left undisturbed at approximately 3.75 P_H. No attempt was made to control reaction values after the seeds were planted, and no subsequent applications of nutrients or phthalate were made. All experiments were run in duplicate, the averages of which are reported in tables I–V. The plants were watered with distilled water, and the growth period ranged from twenty to thirty-five days.

Conclusions

1. Potassium acid phthalate in concentrations of 1000 or more ppm is decidedly harmful to young tomato plants grown in sand and water cultures.
2. As measured by dry weight increases of plants grown in sand cultures, its toxic action is appreciable in concentrations of 500 ppm or less.
3. When applied to soil cultures the opposite effect may occur, and the addition of the phthalate prove beneficial to the growth of the plants.
4. Barley plants give evidence of being somewhat more susceptible to phthalate injury than tomato plants.

The writer desires this opportunity to express his indebtedness to Dr. C. A. SHULL and Dr. S. V. EATON of the Department of Botany for continued interest and helpful advice throughout the course of the experiments.

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FACTORS GOVERNING SEASONAL CHANGES IN TRANSPIRATION OF ENCELIA FARINOSA

EDITH B. SHREVE

In a previous paper[1] it has been shown that the desert perennial *Encelia farinosa* possesses some means of reducing its ratio of transpiration to evaporation (T/E) during the months in which aridity is increasing in its native habitat in southern Arizona. Attention has been called to the fact that the plant thus obeys LE CHATELIER's theorem.[2] The evaporative power of the air is twice as great during May and June as during the cool and more humid months of January and February, while *Encelia* loses only 1.4 times as much water per unit area in the latter months as it does in the former. This investigation was undertaken with the hope of discovering the mechanism of the reduction in relative transpiration.

The plant has two distinct types of leaves, a mesophytic form which is present during the cool months, and a smaller xerophytic one which follows the disappearance of the other form. The mesophytic form is sometimes almost glabrous, while the xerophytic form is always covered with a thick mat of hairs, shown in the illustrations in my, previous paper. In order to ascertain whether or not the leaf tissue alone accounts for the greater resistance to water loss by the xerophytic form, disks were cut from corresponding positions in the two types of leaves, and the water loss measured under the same external conditions. Samples were always taken just before sunrise.

A combination of air temperature and humidity was selected, lying about midway between the two seasonal extremes, and these conditions were maintained as closely as possible in a closed glass chamber. An apparatus was designed for exposing both sides of the disks to the air in such a way that evaporation from the cut surfaces was avoided. Circular holes smaller than the disks were cut at regular intervals in rectangular pieces of aluminum sheeting; the leaf disks were placed over the holes between two sheets and the sheets screwed together. These disk mountings were then arranged so that both sides of the disks were exposed to the air in a vertical position. While weighings were being made, an air-tight cover was fitted over the mountings to prevent loss of water. Eight mountings, each containing eight disks, were then arranged with equal spacing in a glass chamber. Other similar pieces of apparatus filled with wet gray plant drying paper and exposed in a similar manner provided a check on the evaporative conditions of the chamber at all times. It is to be noted that the water loss from the leaf disks was not true transpiration as it occurs in nature. The influence of the water relations of stems and roots has been eliminated, and thus it is possible to compare directly the resistance to the evaporative power of the air offered by the different types of leaf tissue.

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[1] SHREVE, EDITH B., Seasonal changes in the water relations of desert plants. Ecology 4:266–292. 1923.

[2] The theorem is stated by PULLING as follows: "Each change in an outer condition that affects a body or system produces in it a change in such a direction that as a result of this change the resistance of the body or system to this outer change is increased."

In table I are given typical results from these experiments. The figures represent averages of eight mountings, the error due to averages being 0.0005. It is seen that when disks of the two types are placed under the same atmospheric conditions, the xerophytic type loses 0.52 times as much as the mesophytic, a result which seems in agreement with the one that might have been expected from an inspection of the leaf structure. An examination of the water contents of the disks on the two dates, however, shows that there is another factor to be considered. On March 14 the water content of the mesophytic type was 3.3 gm. per gram of dry weight, and the xerophytic type on May 14 was only 1.2 gm. On March 27 the mesophytic leaves were still on the bushes, but their water content had decreased to 2.3 gm. The water loss on March 27 was 0.84 that of the earlier date.

In order to find the effect of a higher water content on the evaporation from the xerophytic disks, branches were cut from the bushes, and the ends immediately placed in water. The branches were covered with bell jars and left to absorb water for 24 hours.

TABLE I

AVERAGES OF RESULTS FROM EXPERIMENTS WITH DISKS OF *Encelia* LEAVES PLACED UNDER IDENTICAL EXTERNAL CONDITIONS; 324 DISKS, 1.6 CM. IN DIAMETER, USED ON EACH DATE

Dates and notes	1	2	3	4	5	6	7	8	9	10	11	12
	Original water content per gm. dry weight (entire leaves)	E_d Water loss from 18 disks (leaves)*	E_a Water loss from 18 disks (paper)	O Original water in 18 disks	M Maximum water in 18 disks after imbibition	Water deficit	Original dry weight 18 disks	Dry weight 18 disks after imbibition	Soluble material lost by imbibition	$E_d \times \frac{M}{O}$	$\frac{E_d}{E_a} \times \frac{M}{O}$	Temperature of cage (F°)
March 14 Mesophytic leaves............	3.3	0.143 (1.00)	0.80	0.735	1.08	0.29	0.22	0.16	0.06	0.210	0.262	81
March 27 Mesophytic leaves............	2.3	0.120 (0.84)	0.80	0.635	1.12	0.35	0.28	0.15	0.13	0.212	0.265	81
May 14 Xerophytic leaves.............	1.2	0.075 (0.52)	0.80	0.590	1.67	0.92	0.41	0.25	0.16	0.212	0.265	81
May 18 Xerophytic leaves from branches that have absorbed water for 24 hours..................	2.3	0.113 (0.79)	0.80	0.685	......	......	0.31	......	......	......	......	81
May 27 Xerophytic leaves from plants irrigated for 10 days........	2.0	0.105 (0.74)	0.80	0.675	1.37	0.61	0.34	0.25	0.09	0.213	0.266	81

Disks were then cut from these leaves and their water loss measured in a manner identical with that used for the untreated leaves. The water loss returned to an amount 0.79 as great as that from the mesophytic leaves on March 14. The water content of these xerophytic leaves had increased from 1.2 to 2.3 gm. The water content of other xerophytic leaves was increased from 1.2 to 2.0 gm. by irrigating for ten days plants growing naturally in the open. Again the water loss for equal areas increased markedly (table I). Thus it is evident that the leaf structure cannot be the main factor in increasing the resistance to water loss by evaporation. The evidence of this experiment is that the water deficit in the leaf is a strong controlling factor. When an attempt to obtain a constant is made by using the water loss for equal areas and the water content based on dry weight the result is negative. If the water loss for equal areas is divided by the actual amount of water in the same areas, an approach to a constant is obtained from the ratios E_d/O, but it is far enough away from a true constant to make it certain that other factors must operate.

Working on the hypothesis that a relation exists between the water absorbing capacity and the water holding ability or retentiveness of leaf tissue, the imbibition of leaf disks for both seasons was measured. The two sets of experiments of course were conducted at the same temperature. The maximum water absorbed by equal areas is given in column 5. Here again there is very evidently some relation, for the greater the absorbing capacity the less the water loss by evaporation; but no true constant appears. If, however, the two factors (original water and maximum water obtained by imbibition) are considered together in the form $E_d \times M/O$, a constant is obtained which is exceptionally good for plant work. This is especially true when it is observed that the third decimal is uncertain. It thus appears that under identical atmospheric conditions the water loss from the leaf tissue of this plant varies directly with the original amount of water present, and inversely with the water absorbing capacity of the tissue, regardless of whether the leaf is of the xerophytic or the mesophytic type.

Further evidence of this relation appears in some preliminary experiments that were originally discarded, either because porous cup atmometers were used instead of felt paper, or because the cage failed to hold a constant temperature. The results of these experiments are given in table II. The water losses from leaf disks at the different temperatures have been reduced to comparable terms by using the response to the evaporative power of the air, E_d/E_a, after the manner of the well known transpiring power ratio T/E. On March 1 and 12 the disks used were of a larger diameter; and cylindrical porous cup atmometers measured the evaporative power of the air. The expression $E_d/E_a \times M/O$ gives the same number for the two dates. On the remaining three dates given in table II the disks were of the same diameter as those in table I, and felt paper measured the evaporative power of the air. Again a constant for these dates appears. If the ratio E_d/E_a is used instead of E_d in table I, the numbers obtained from the expression $E_d/E_a \times M/O$ are the same for all dates with the exception of the first two in table II. These last could not agree with the others because the atmometers give a different unit for the evaporative power of the air; but it is significant that they agree with each other. The expression may now be stated thus: the response to the evaporative power of the air shown by isolated pieces of the leaf tissue varies directly with the original water content of the tissue and inversely with the maximum water content obtained by its imbibition of water in vitro.

It must be remembered that this expression represents the relations existing in an isolated piece of leaf tissue which cannot draw water from the rest of the plant. In the entire plant system the complications of the water content of stems and roots and their water absorbing and retaining capacity, as well as the resistance of water passage, enter into the final resultant of the drought resistance of the whole plant. These experiments show the internal factors operating in the leaf tissue alone. In my former paper it was shown that the transpiration per unit area for a 24 hour period for *Encelia* (T) is a function of at least two external factors, the evaporation power of the air (E) and the water content of the soil (S). The expression $T/(E \times S)$ so nearly approached a constant value that the conclusion was drawn that these two factors represented the major external influences regulating transpiration. Attention was called to the fact, however, that the results showed that still other conditions must be operative, and that these were most probably internal. The ratio of the evaporative power of the air to soil water content (E/S) affects the water content of leaf tissue, which in turn affects the water absorbing capacity of the tissue. Thus the two expressions $T/(E \times S)$ and $E_d/E_a \times M/O$ partly indicate the resistance of *Encelia* to drought and its accord with LeChatelier's theorem.

The changes in original water content of leaf tissue seem to be accounted for by the failure of the plant to maintain its water balance with increasing aridity; but the causes for the changes in absorbing capacity are evidently more difficult to determine. An examination of the values obtained for the dry weights before and after imbibition throws some light on the mechanism of the changes in water absorbing capacity. The dry weights for eighteen disks, that is, for equal areas, vary considerably with the conditions of the experiments, the greatest difference being between March 14 and May 14 when the xerophytic disks have a value nearly 1.9 greater than the mesophytic.

TABLE II

Averages of experiments with disks of *Encelia* leaves under slightly different external conditions

Dates and notes	1	2	3	4	5	6	7
	E_d Water loss per hour (leaf disks)	E_a Water loss per hour (atmometer)	O Original water in 18 disks	M Maximum water in 18 disks	$\frac{E_d}{E_a}$	$\frac{E_d}{E_a} \times \frac{M}{O}$	Diameter disks (cm.)*
March 1 Mesophytic leaves first 2 hours	0.16	0.64	0.94	1.25	0.250	0.332	2.6
March 12 Mesophytic leaves first 2 hours	0.18	0.83	0.87	1.34	0.217	0.333	2.6
March 12 Mesophytic leaves first hour	0.125	0.69	0.735	1.08	0.181	0.265	1.6
May 13 Xerophytic leaves first hour	0.074	0.78	0.590	1.67	0.095	0.268	1.6
May 28 Xerophytic leaves first hour	0.120	0.91	0.675	1.37	0.132	0.268	1.6

* Area exposed to air same in all cases.

When these disks were left in distilled water for 24 hours, soluble material diffused into the water; but the amounts were different for the different dates. The less the original water content of the leaf the greater the amount of soluble material that diffused into the water. The dry weights of the disks after imbibition show one constant value for the mesophytic form and a larger one for the xerophytic. In the xerophytic leaf the palisade cells are shorter and more closely packed than in the mesophytic, where there are large intercellular spaces. Thus disks of equal areas would include more cells and consequently more insoluble material than the mesophytic disks, and the difference of 0.15 and 0.25 in the dry weights might be accounted for.

The difference in the diffusible material offers an interesting hypothesis. It may be supposed that, as the plant loses water by excessive transpiration, reversible chemical changes occur that cause certain materials to give up water of combination and to become temporarily insoluble. When more water is available, these substances again undergo change into a soluble form, and are carried by diffusion into other parts of the plant. The results of these experiments agree with this hypothesis. When the plants with xerophytic leaves were irrigated, the original weight decreased from 0.41 to 0.34 gm., and when the cut branches were left in water for 24 hours, the decrease was from 0.41 to 0.31 gm.; while natural desiccation of the mesophytic leaves from 3.3 to 2.2 gm. was accompanied by an increase in dry weight from 0.22 to 0.28 gm. Thus it seems highly probable that the increase in dry weight for equal areas which accompanies a decrease in the water content is the cause of the increase in the water absorbing capacity.

In times of drought the leaves and stems are filled with a dark brown, viscous sap which is almost unfilterable, and which is probably a colloid solution. This liquid oozes from every wound under drought conditions, but is absent during the moist season. When the brown sap is expressed from the leaves and heated to 80° C., a bulky gelatinous precipitate is thrown down. Possibly this contains the material which is rendered insoluble by loss of water, and which is responsible for the increase in water absorbing capacity shown by the tissue of low water content. The amount of precipitate obtained from xerophytic leaves taken from irrigated plants was less than that obtained from the xerophytic leaves of lower water content, a fact in agreement with the theory suggested.

Summary

1. In a former paper it has been shown that *Encelia farinosa*, a desert perennial having a mesophytic form of leaf in the cool moist months and a xerophytic form in the arid season, reduces its response to the maximum evaporate power of the air in June to about one-fifth the January value.

2. The difference in the anatomical structure of the mesophytic and xerophytic leaves does not account for the greater resistance to water loss in the arid season.

3. When disks of equal areas are cut from the two types of leaves immediately before sunrise and placed under identical external conditions, the water loss from them is found to vary inversely with the total imbibitional capacity and directly with the original water content. Even though the external temperature varies as much as 10° F., the expression $E_d/E_a \times M/O$ gives a value which approaches a constant to an amount remarkable for work with water relations of plants.

4. The total imbibitional capacity of both types of leaves varies with the original water content and the dry weight.

5. The lower the original water content, the greater the amount of soluble material that diffuses into the water which surrounds imbibing disks. The dry weights after imbibition have one constant value for mesophytic leaves and another for xerophytic ones.

6. The ratio of evaporation to soil moisture affects the water content of leaf tissue; this in turn affects the imbibitional capacity and resistance to water loss of leaf tissue. The changes in imbibitional capacity are accompanied by changes in the amount of soluble material in the tissue. These changes in diffusible material are presumably the resultants of reversible chemical changes which take up or give up water according to the amount available.

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DEVELOPMENT OF SEED OF CROTALARIA SAGITTALIS

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(WITH PLATES XXX, XXXI)

This plant was selected for study as typical of those plants in which the embryo absorbs practically all the endosperm and nucellus, and completely fills the one seed coat at maturity. The mature seed, therefore, is composed of a very large embryo and single seed coat. The food for the early support of the seedling is stored in the two very fleshy cotyledons. The seed is of the same type as the bean, but is smaller and much more easily handled for a study of this kind. None of the material collected was young enough to show the development of the embryo sac. The youngest stage observed was an eight-celled sac in which the antipodals were disintegrating and the polar nuclei uniting (figs. 1, 2). It is well known that in the members of this family already studied the antipodals disintegrate very early. It is safe to assume that the development of the embryo sac in this species is in line with that of other species of the Leguminosae. Immediately following fertilization, both the seed and the embryo sac enlarge very rapidly, and the former feels much like a small bladder when pressed between the thumb and finger. This is the result of the very rapid formation of nucellus and its equally rapid disintegration to form the endosperm, which is evidently active in the feeding of the embryo. During this period the sac is lined with a well defined endosperm, and the center is probably filled with liquid food which serves for the immediate use of the growing embryo. The seed, therefore, is almost full size while the embryo is very young and small (figs. 3, 13, 30). The ovules are attached alternately on either side of a ridge along the dorsal side of the ovary (figs. 4, 5); that is, the side corresponding to the midrib of a foliar organ.

POLLEN TUBES.—The pollen tubes are very numerous and prominent. They penetrate the upper end of the ovary cavity and follow the ridge to which the ovules are attached (figs. 4, 5). They are so intimately associated with the cells of the carpel in this region that it is frequently impossible to distinguish them (figs 6, 7). The tips of the pollen tubes along the edge of the ridge turn into the ovarian cavity (figs. 6, 7). The number of pollen tubes is so much in excess of the number of ovules that only one in many ever reaches an ovule, the others disintegrating (fig. 8). The entrance of the pollen tube through the micropyle and into the embryo sac is clearly visible, the remnant of the tube usually persisting for a considerable time after fertilization (figs. 10–13, 16.) In one case the pollen tube had formed three branches, only one finding its way into the embryo sac (fig. 12). It is very generally recognized that the pollen tube is parasitic on the ovary of the sporophyte, but it is not generally considered as parasitic on the female gametophyte. In this study one case was observed in which the pollen tube penetrated the embryo sac and destroyed its contents (fig. 9). This has previously been observed by the writer in the case of *Passiflora adenophylla*.[1] Why the pollen tube occasionally behaves in this manner the writer does not attempt to answer.

NUCELLUS.—The nucellus at first is composed of small cells which are very compact at time of fertilization (figs. 13, 25, 26). It increases very rapidly, the cells increasing both in number and size and becoming very vacuolate. Those cells lying next to the endosperm disintegrate very rapidly, so that there is never a large amount of nucellus at any one time (fig. 30). A small amount of nucellus persists in the region of the hilum after maturity.

FERTILIZATION.—The disintegration of the antipodals and the enlargement of the embryo sac begin with or just before the entrance of the pollen tube into the sac (figs. 1, 3, 30). The polar nuclei unite at this time and apparently just before the entrance of the pollen tube (fig. 2). It is very doubtful whether triple fusion occurs in this species. The relationship of the entrance of the pollen tube into the embryo sac to the behavior of the synergids was not observed, but it is evident that both synergids disintegrate at about the time of the entrance of the tube (fig. 10). The actual union of the sperm nucleus and the ovum was not observed; but the tip of the pollen tube just penetrating the sac was observed repeatedly (figs. 10–13, 16). In one case the tip of the tube contained two well defined bodies, although the embryo was well advanced (fig. 11). These two bodies had the appearance of two sperm nuclei. If they were sperm nuclei, the embryo was evidently forming parthenogenetically. On the other hand, these two bodies may have been fragments of a single disintegrating nucleus. In that case, one sperm nucleus may previously have united with the egg.

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[1] COOK, M. T., Notes on the embryo sac of *Passiflora adenophylla*. Bull. Torr. Bot. Club **36**:273–274. 1909.

EMBRYO.—The development of the embryo, like that of many other of the legumes, is extremely variable. The first stage of development is in the form of a sphere (fig. 10), but the divisions may be very irregular (figs. 11, 12). In some cases the nuclei appear to divide well in advance of the formation of walls (fig. 11). In most cases the divisions are regular and result in the formation of a spherical or more often of an elongated embryo, which gives rise to the embryo proper and the suspensor (figs. 14–16). The suspensor is comparatively slow in its development. At first it is composed of a few large cells (fig. 15), but finally develops into a prominent organ bearing a much smaller embryo proper (fig. 16). It is extremely variable, in some cases being large and smooth (fig. 16), and in others covered with projections (figs. 17, 18). These projections are short, unicellular, trichome-like elongations from surface cells. This development of the embryo is very similar to that described by GUIGNARD[2] for *Spartium junceum*. He has also observed outgrowths on the suspensor of some species. Following this period of development, the embryo proper enlarges, and very early shows a differentiation leading to the development of the cotyledons (fig. 19). The cotyledons are well advanced; in fact the embryo has almost reached its complete growth before there is any evidence of cell division for the formation of the plumule (fig. 20). The radicle starts to develop a little later than the cotyledons (fig. 21). The suspensor persists until very late, but the embryo has undergone such enlargement that it is comparatively small at this time.

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[2] GUIGNARD, M. L. Recherches d'embryogénie vegétale comparée. Ann. Sci. Nat. Bot. 12:5–166. 1881.

Polyembryony was observed in two cases (figs. 22, 23). In one case there were two and in the other four embryos. GUIGNARD observed cases of two and three embryos in the sacs of *Mimosa Denhartii*, and was of the opinion that the extra ones were derived from synergids. In the case of *C. sagittalis* the writer was unable to determine this point, but the early disintegration of the synergids makes it improbable that the extra embryos came from this source. They are most likely derived as a result of a splitting of the primary embryo derived from the egg.

ENDOSPERM.—The endosperm nucleus divides before the first division of the embryo and develops very rapidly. It forms a delicate lining for the entire embryo sac, with considerable masses immediately surrounding the embryo and at the opposite end of the sac (figs. 10–13, 16, 24, 26). The nuclei are abundant and never in more than one layer, except immediately surrounding the embryo and at the opposite end of the sac. The endosperm practically disappears with or just before the last of the nucellus cells and the inner cells of the seed coat (fig. 29).

SEED COAT.—A single seed coat is formed, which at first consists of four layers of cells except in the micropylar and hilum regions, where they are more abundant (figs. 13, 25, 26). These cells are thin and flat when young, but those of the outer layer soon tend to thicken and become more or less cubical (fig. 26). This tendency to thicken continues until the cells become columnar, the long axis being at right angles to the surface of the ovule (figs. 27–29). The cells of the second and third layers tend to become cubical, and the innermost layer of cells disintegrates (fig. 27). This is followed by a continued elongation of the cells of the outer layer, the formation of intercellular spaces between the cells of the second layer, and a disintegration of the cells of the third layer (figs. 28, 29). In the meantime the cuticle has become highly developed, and the walls of the cells of the two remaining layers stain as though highly cutinized. Fragments of the two inner layers of cells, of the nucellus, and of the endosperm may be seen at this time (fig. 29).

CLEAVAGE PLANE.—The formation of the cleavage plane in the hilum is very marked between two layers of cells which are richer in protoplasm and stain more deeply than the surrounding cells (fig. 31). The cells of these two layers tend to become cubical and then columnar, the same as the outer layer of the seed coat (figs. 31–33). The line of demarcation becomes more and more conspicuous, and exposed surfaces of the two layers of cells become cutinized. Long before the seed is mature, the two layers can readily be separated by a slight pressure on the cover glass.

This work was done at the New York Botanical Garden during a period of leave of absence from Rutgers College.

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EXPLANATION OF PLATES XXX, XXXI

FIG. 1.—Eight-celled embryo sac; antipodals disintegrating and polar nuclei uniting.

FIG. 2.—Eight-celled embryo sac; antipodals have disappeared and polar nuclei united.

FIG. 3.—Diagram of ovule with eight-celled embryo sac.

FIG. 4.—Diagram of inner dorsal side of ovary, showing ridge, points of attachment of ovules, and entrance of mass of pollen tubes (*pt*).

FIG. 5.—Cross-section of diagram of ovule, showing one ovule and dorsal ridge.

FIG. 6.—Upper part of ovary, showing entrance of pollen tubes.

FIG. 7.—Part of same under higher power.

FIG. 8.—Disintegrating pollen tubes that have failed to reach ovules.

FIG. 9.—Pollen tube destroying contents of embryo sac (semi-diagrammatic).

FIG. 10.—Very young embryo; tip of pollen tube attached; endosperm well advanced.

FIG. 11.—Embryo; prominent endosperm; tip of pollen tube showing two bodies.

FIG. 12.—Embryo; endosperm; tip of pollen tube which has branched.

FIG. 13.—Part of ovule, showing seed coat, nucellus, micropyle, embryo, and endosperm; *pt*, pollen tube.

FIG. 14.—Embryo.

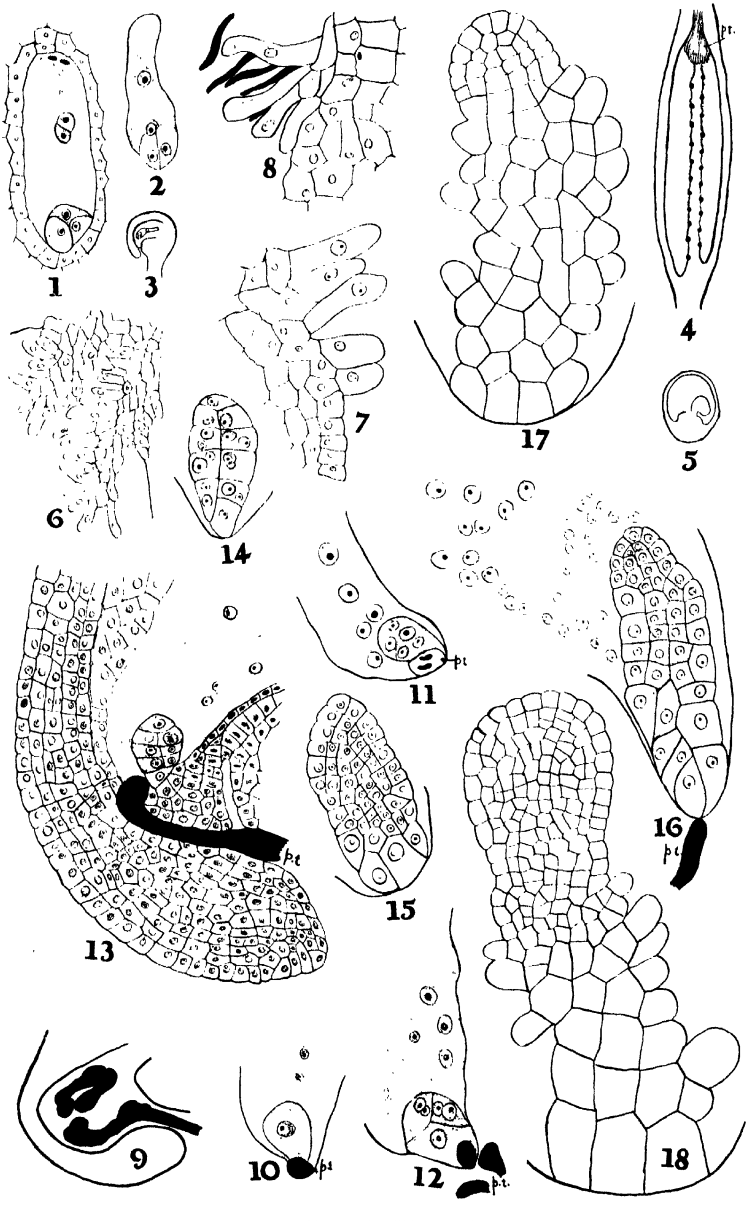
FIG. 15.—Embryo.

FIG. 16.—Embryo and endosperm; *pt*, pollen tube.

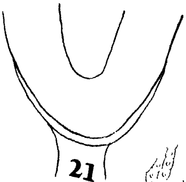
FIG. 17.—Embryo and prominent suspensor.

FIG. 18.—Embryo and prominent suspensor.

FIG. 19.—Part of embryo, showing origin of plumule.



COOK on CROTALARIA



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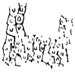

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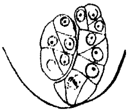

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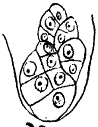

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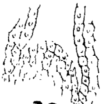

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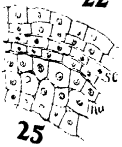

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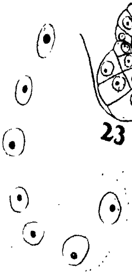

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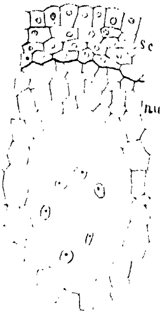

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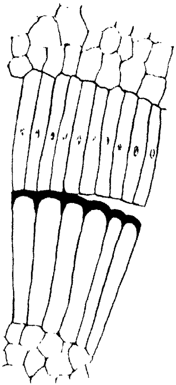

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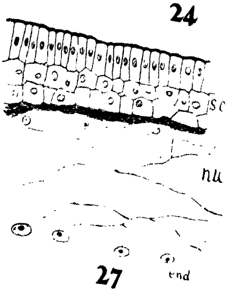

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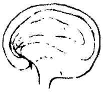

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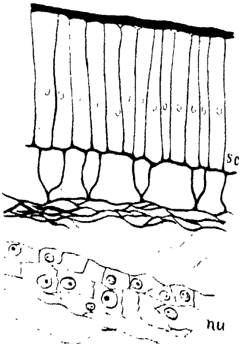

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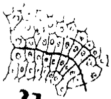

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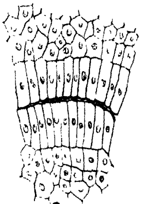

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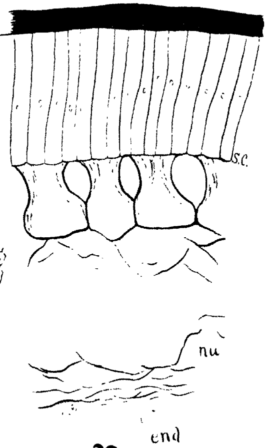

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FIG. 20.—Part of embryo, showing origin of plumule (older than fig. 19).

FIG. 21.—Part of embryo, showing origin of radicle (diagrammatic).

FIG. 22.—Polyembryony.

FIG. 23.—Polyembryony.

FIG. 24.—Endosperm in end of sac opposite embryo.

FIG. 25.—Part of ovule, showing four layers of cells of seed coat (*sc*), and three layers of cells of nucellus (*nu*).

FIG. 26.—Part of ovule, showing four layers of cells of seed coat, part of nucellus, and endosperm lining embryo sac; cut at right angles to fig. 25.

FIG. 27.—Three layers of cells of seed coat, fourth layer disintegrating; nucellus and endosperm.

FIG. 28.—Two layers of cells of seed coat, third layer disintegrating; nucellus disintegrating.

FIG. 29.—Two layers of cells of seed coat; disintegrating nucellus and endosperm.

FIG. 30.—Diagram of ovule about same age as fig. 13.

FIG. 31.—First stage in formation of cleavage plane.

FIG. 32.—Second stage in formation of cleavage plane.

FIG. 33.—Third stage in formation of cleavage plane.

CYTOLOGICAL AND PHYSIOLOGICAL CHANGES IN VICIA FABA IRRADIATED WITH RÖNTGEN RAYS[1]

HIDEO KOMURO[2]

(WITH ONE FIGURE)

In 1922 the writer published a preliminary note on the cells of *Vicia Faba* modified by Röntgen rays, and their resemblance to tumor cells.[3] Because of this resemblance, these experiments have been repeated, using different kinds of X-ray tubes and a race of *Vicia Faba* called "Wasesoramame," which was obtained from Nihon-Noen in Tokyo. Microscopical observations cannot be made on the material of the 1923 experiments in the near future, so that at this time the results of the cytological experiments of 1922 are reported briefly, with observations on the physiological aspects of the 1923 experiments. Methods will be explained in detail in a later complete paper.

The Röntgen tube used was OKURA's gas tube with tungsten anticathode, the hardness of which was ± 10.5° of WEHNELT. Seedlings in water in a Petri dish were placed under the X-ray bulb, the distance between the anticathode and the material being about 30 cm., and were irradiated with a current of 2.0–2.5 milliampères for one hour.

Cytological observations

Beginning 1.5 hours after irradiation, degeneration phenomena were observed as follows.

Preparations made 1.5 hours after irradiation: Vacuolization of cytoplasm apparent; chromatolysis clearly evident, only nuclei with distinct membranes being present; all observed mitoses abnormal.

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[1] Preliminary note on cytological experiments of 1922 and observations on physiological aspects of experiments of 1923.

[2] Traveling Fellow from the Department of Education of the Imperial Nipponese Government.

[3] Bot. Mag. Tokyo 36: no. 424. 1922.

Preparations made 6 hours after irradiation: Nuclear membranes not visible, owing to degeneration; nuclear contents stained dilute grayish black by haematoxylin, with numerous small granules stained deeply with the same dye (feinkörnige dichte Hyperchromatose); pyknotic and karyolytic conditions frequent; abnormal binucleate cells present. These changes are not observed in the control preparations.

Preparations made 9 hours after irradiation: Vacuolization of cytoplasm manifest; karyolytic and pyknotic conditions prevalent; escaped nuclei very often seen in cytoplasm. There are many nuclei in the condition of "feinkörnige dichte Hyperchromatose" and of "Kernwandhyperchromatose" (chromatic substance adhered to the nuclear membrane), while a degeneration of the nuclear membrane and a deformation of the nucleus are of common occurrence. Many abnormal binucleate cells are found, as well as many others which appeared to be approaching this condition, for it seemed evident that the mitotic process was interfered with in the prophase stages, with the result that the nuclei had become constricted and irregularly divided. The process often bears a striking resemblance to amitosis. Many giant nuclei stained deeply by haematoxylin are found, and also multinucleolar nuclei. These alterations cannot be seen in the controls.

In the root tips of seedlings, these cytological changes occurred within 1.5 hours after exposure for one hour to Röntgen rays from a tungsten anticathode (gas tube). Nine hours after irradiation, binucleate cells, giant nuclei, and multinucleolar nuclei were found, and these changes resemble those described in the writer's preliminary note of 1922. Besides these conditions, various stages of degeneration were manifest, only abnormal mitotic figures being found 1.5 hours after irradiation.

Physiological observations

Before leaving Nippon the writer made five experiments for the purpose of examining the effect of soft rays from a molybdenum anticathode of a Coolidge tube. Tips of radicles of seedlings of *Vicia Faba* were preserved for the cytological investigation to be made after the physiological experiments were finished. The seeds used for these experiments were of the "Wasesoramame" race. In the irradiation a water cell of aluminium was used.[4]

Experiment I

Two hours' irradiation of air-dried seeds (tube dist. ca. 15 cm.; 7°–8° (W.); 2d cur. 1.6–1.7 m.a.; temp. 16°–17° C.).[5]—Immediately after irradiation the seeds were sown in pots of sand and placed in a greenhouse. The treatment of seeds after irradiation was the same in all the experiments.

Seven days later ($162\frac{2}{3}$ hours after irradiation) the radicles of the irradiated seeds had reached a length of 2.2, 3.7, 3.8, 3.9 and 4.1 cm., and their tips were yellowish green. Fourteen days after irradiation, the length of the radicles varied between 4.5 and 7 cm., and some of them bore lateral roots. Two hours' irradiation from a molybdenum anticathode does not deprive the air-dried seeds of the power of germination.

Experiment II

Two hours' irradiation of seeds steeped 67 hours (2d cur. 1.6 m.a.; heat. cur. 4 amp.; 4.6°–7.4° C.).—The results are shown in table I, which shows the severe inhibitory effect of two hours' irradiation on the development of steeped seeds. The tips of radicles from the irradiated seeds were anomalous in form and in a somewhat necrotic condition.

Experiment III

Intermittent two hours' irradiation of seeds steeped 67 hours.—Seeds were exposed to rays for one hour under the same condition as in experiment II, and 45 hours later a secondary irradiation of one hour was made (4.6°–7° C.). The results are shown in table II. On the twelfth day after irradiation, the tips of the radicles became stumpy and harder than the controls, and blackish brown or black in color. Most of them were malformed and in a necrotic condition.

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[4] Komuro, H., Studies in the effect of Röntgen rays upon the development of *Vicia Faba*. Jour. Coll. Agric. Imper. Univ. Tokyo **8**: 1923 (p. 284). The irradiation was made by the writer under the direct supervision of the late Professor K. Fujii at the Electrical Laboratory of the Agricultural Experiment Station of the Department of Agriculture and Commerce, Nishigahara, Tokyo.

[5] Abbreviations: tube dist., distance between anticathode of X-ray tube and seeds to be irradiated; 2d cur., secondary current; heat. cur., heating current; m.a., milliampères; amp., ampères; W., hardness of X-ray tube as shown by Wehnelt's measuring instrument; temp., temperature of place in which seeds were irradiated.

Experiment IV

Intermittent two hours' irradiation of seeds steeped 43 hours.—Seeds were exposed to rays for one hour (heat. cur. 4 amp.; 2d cur. 1.7 m.a.; tube dist. ca. 11 cm.; temp. 8.2°–10° C.), and 47⅔ hours later they were again exposed for one hour under the same conditions (temp. 6.8°–8° C.). In these experiments special care was taken to expose the seeds similarly to the rays, and, in the case of intermittent irradiation, to minimize the loss of water from the steeped and irradiated seeds.

TABLE I

HOURS AFTER IRRADIATION	CONTROL		X-RAYED	
	Length (cm.)		Length (cm.)	
	Radicle	Plumule or shoot	Radicle	Plumule
92⅓	0.6–1.8	....	0.6–1.0	....
162⅔	5.5	4.2	2.0	3.7
	7.3	3.6	0.7	3.0
	4.5	4.3	0.3	Does not come out of seed coat
	9.1	5.2	....	

TABLE II

NO. OF DAYS AFTER SECOND IRRADIATION	CONTROL		X-RAYED	
	Length (cm.)		Length (cm.)	
	Radicle	Plumule	Radicle	Plumule
5	4.3		0.7	
	3.9		0.7	
	1.3		....	
10	Young plants having many lateral roots	Length above soil 3.5–5.0	1.3	1.0
			1.4	0.7
			2.3	1.0
			1.7	Does not come out of seed coat
			0.5	

Sixteen days after irradiation, X-rayed radicles had reached 0.3, 1.4 (three), 1.5, 1.6, 1.7 (two) cm. in length (average 1.36). They were stumpy, hard, blackish brown or black, and were in a necrotic condition, while the control plants developed to the stage having 3–5 nodes above the soil. Fig. 1 shows the state of growth sixteen days after irradiation, the tips of the radicles having been cut off for the purpose of cytological investigation.

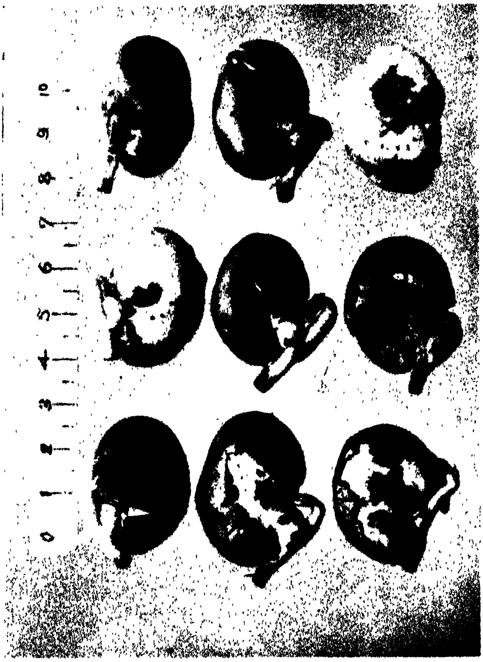

FIG. 1

Two hours intermittent irradiation produced more marked effects than did the continuous irradiation of the same duration, and the seeds thus treated ceased to grow at almost the same stage as is shown in fig. 1. Even in steeped seeds, two hours' irradiation could not deprive them of the power of germination. They could develop to a certain stage, however, verifying the writer's paper of 1923 (p. 287[1]).

Discussion

The writer has previously pointed out the resemblance of cytological changes caused by the irradiation with Röntgen rays to those appearing in tumor cells, and he has repeated the experiments on the effect of these rays, using the seedlings of another race of *Vicia Faba*, "Wasesoramame." As early as 1.5 hours after irradiation the degeneration process is under way, and nine hours after exposure one may observe various stages of degeneration. These resemble the conditions described in the writer's previous paper, and closely approximate those noted by him and other investigators in tumor cells. Although a cytological investigation of the material obtained this year could not be made, the physiological results were almost the same as in the formerly reported cases. This was especially true with regard to the effects of intermittent irradiation, which has never been employed by other investigators using plants. In the therapeutic use of Röntgen rays they were usually allowed to act on tissue intermittently. From these results it may safely be inferred that this intermittent use of X-rays in all probability will be harmful to the human body, although various filtering methods may be used; for X-rays may be summed up to harmful doses by repeating the exposure. Moreover, we must take the nature of the X-rays (that is, soft and hard) and the doses it is possible to give into consideration. The severe effect of rays from a tungsten anticathode (hard rays) at small milliamperages in cytological experiments should be borne in mind, and that of the physiological effects caused by soft rays from a molybdenum anticathode.

The practical bearing of the results of this investigation should be emphasized. The results of cytological and physiological experiments, together with the case of Yamagiwa and Ichikawa's artificial carcinom,[6] and the carcinom caused by the chronic stimulation,[7] and that of the Röntgencarcinom reported by Bichler[8] have led the writer to the conclusion that the therapeutic use of X-rays will be dangerous and of questionable value, because they may be expected to cause cytological alterations resembling those in malignant tumor cells.

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[6] Yamagiwa and Ichikawa, Mitt. d. Med. Fak. Kais. Univ. Tokyo. 15:1915, 17:1917, 19:1918, 22:1919. Amer. Jour. Can. Res. 11:1917.

[7] Boveri, Th., Zur Frage der Entstehung maligner Tumoren. 1914 (p. 37).

[8] Bichler. Zur Kasuistik des Röntgencarcinoms. Wiener klin. Wochens. 1914. Nr. 26.

The writer is grateful to acknowledge his indebtness to the late Professor K. FUJI, who has kindly helped in making the exposures to rays, and to Professor L. W. SHARP, who has looked over the manuscript. Thanks are also due to the Morimura Hômei Kwai and to Mr. K. OHNISHI for defraying the expenses of the present research.

BOTANICAL LABORATORY
CORNELL UNIVERSITY

BRIEFER ARTICLES

GEORGE LINCOLN GOODALE

(WITH PORTRAIT)

Professor GOODALE was born August 3, 1839, at Saco, Maine. He graduated from Amherst in 1860, and received the degree of M.D. from Harvard in 1863. His first teaching position was at Bowdoin (1869–1872), where he was professor of "materia medica and natural science," a good illustration of the range of teaching demanded of a professor of science in those early days. In 1872 he became instructor in botany at Harvard; in the following year he was appointed assistant professor; and in 1878 became professor of botany, a position which he held until his retirement in 1909, when he became emeritus professor. He died April 12, 1923, in his 84th year.

Professor GOODALE was a notable teacher. It was the good fortune of the writer to work under him for two summers, during one of them as his laboratory assistant. He was certainly gifted as a remarkably clear and attractive lecturer, organizing and presenting his subject in a way that his pupils could never forget. As many students in those days remarked, he "really made botany interesting," in its early excursions beyond taxonomy. He was not only a great teacher, but also an unusually efficient organizer. Through his ability as a teacher and an organizer he was able to relieve Dr. ASA GRAY of the tedious routine of elementary instruction and many administrative cares. Associated with the abilities mentioned was a very kindly personality, so that his relations with students were very helpful and stimulating.

While his chief interest was in teaching and in building up a botanical equipment, his own field of work was in economic botany, in the improvement of useful plants and their products. In this connection, he was especially interested in the Harvard Experiment Station in Cuba.

Professor GOODALE will stand as probably the greatest teacher of botany in the early days, when botany was beginning to extend its range, and laboratory work began to be a feature. He was a pioneer in this extension, which has led to the great differentiation of the subject today.—J. M. C.

CURRENT LITERATURE

BOOK REVIEWS

The Cactaceae

The Carnegie Institution of Washington is to be congratulated on the completion of its monograph on the Cactaceae.[1] This large, important, and peculiar family of plants, almost wholly American, had long been in need of a thorough revision. The work was undertaken by N. L. **Britton** and J. N. **Rose**. While the major part of the investigation was financed by the Carnegie Institution, considerable aid was given by the New York Botanical Garden, the United States National Museum, and the United States Department of Agriculture. The New York Botanical Garden gave the use of four greenhouses, financed several field trips, and prepared most of the paintings used for the colored plates. The Department of Agriculture furnished and equipped a large greenhouse, where at one time have been assembled about 5000 plants.

This investigation is undoubtedly the most comprehensive piece of cooperative work that has even been undertaken in any botanical field. The results have been epoch-making, and will for a long time remain the standard in this group of plants. The investigation has led, not only to the publication of four elaborate volumes, but to the assembling of two great cactus herbaria, one in the Smithsonian Institution and the other in the New York Botanical Garden. The collection in Washington occupies 48 standard cases with 1152 compartments. It contains representatives of all the genera and most of the species described in this work. The collection in the New York Botanical Garden, although perhaps not so large, possesses all the more important series.

The Carnegie Institution took up this project in 1912, but it is now twenty years since the authors began the study of the Cactaceae by field, greenhouse, and herbarium work. Perhaps no other group of plants presents so many difficulties to the systematic botanist as the Cactaceae, chiefly because it is very difficult to make herbarium specimens for preservation, and because the early students of this family preserved very few of their types. In other cases the types had been destroyed or lost. Thus many old descriptions had been incorrectly interpreted, plants had been wrongly identified, and such errors had been perpetuated. The authors, fully aware of this, made it the basis of their work to re-examine all the original descriptions.

As most of the Cactaceae had been first described by European botanists, **Rose** went to study all the important herbaria and collections of living plants in Europe in 1912, visiting England, France, Germany, Belgium, Switzerland, and Italy. Extensive field operations in the cactus regions of both Americas were undertaken by the authors and their assistants, and many naturalists have voluntarily helped to collect material. Thus in 1913, 1914, and 1915 the authors visited the West Indies; in 1914 Dr. and Mrs. ROSE went to Jamaica, Panama, Peru, Bolivia, and Chile; in 1915 ROSE explored the cactus regions of Brazil and Argentina; in 1916 those of Curacao and Venezuela; and in 1918 those of Ecuador. Dr. and Mrs. BRITTON have repeatedly visited the West Indies, and SHAFER collected for six months in the desert regions of Uruguay, Paraguay, Argentina, and Bolivia. All the cactus deserts of Mexico and the southern and southwestern United States had been widely explored before 1913 in repeated excursions by ROSE. No other study of the Cactaceae has been based on such an extensive and comprehensive collection of living and dried material, accompanied by notes and photographs made in the native haunts of these remarkable plants.

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[1] **Britton**, N. L., and **Rose**, J. N., The Cactaceae. Descriptions and illustrations of plants of the Cactus family. 4to. 4 vols. 1919–1923.

The last monograph of the Cactaceae was published by SCHUMANN in 1898, to which he added a supplement in 1903. His systematic disposition of the cacti was, with some alterations, chiefly that of SALM-DYCK of 1849, who in his day had been the great authority on this family. This classification was in many cases based on external vegetative characters, as flowers and fruits of most of the plants were then unknown. Efforts to use characters taken chiefly from the reproductive organs were first made by ENGELMANN, LEMAIRE, WEBER, and the writer of this review, but none of them had as rich material or as vast field knowledge of the entire family at their command as had BRITTON and ROSE.

The result of this comprehensive study is now presented in four large quarto volumes, abundantly illustrated by colored plates, photographs, and drawings.

In 1903 SCHUMANN recognized about 800 species, which he grouped under 20 very heterogeneous genera; while BRITTON and ROSE describe 1255 species under 124 genera. The number of genera may seem to be too large, and one might feel inclined to unite two or three here and there, yet the number would probably not be diminished, since one might also segregate a new genus from some of those described. As a rule, the genera are founded on characters of flowers and fruit, with which the vegetative characters and the geographical distribution agree. In fact, I regard the establishment of these new genera as the most important contribution, since they show in a very concise way the development which the family exhibits.

The authors maintain the three principal tribes already recognized by former writers: PERESKIEAE, OPUNTIEAE, and CEREAE. The first volume treats of the first two tribes. The tribe PERESKIEAE contains but one genus, *Pereskia*, with 19 species. These plants look like ordinary trees or shrubs with large flat leaves, rather than cacti, for the spines are more or less concealed. The tribe OPUNTIEAE is much more varied, having seven genera of various habit, some resembling *Pereskia*, with well formed flat leaves. The bulk of this tribe is included in the genus *Opuntia*, which comprises 263 species, or 117 more than were known to SCHUMANN. It is the largest genus of the family, covering its whole range, and extending farther than any other genus both north and south toward the colder regions. Many are native to the United States, some extending as far north as British Columbia in the west, and Nantucket, Massachusetts, in the east. In its development this genus shows a marked uniformity in its reproductive organs, but a wide range in its vegetative characters. It has always been the stumbling block to the cactus student, who will therefore greatly appreciate this clear treatment, which will help to overcome difficulties in identifying these plants.

Volumes II–IV present the tribe CEREAE, which comprises the greatest variety of genera and species, and is divided into eight natural subtribes.

Volume II contains the subtribes CEREANAE and HYLOCEREANAE, which were formerly included in the unnatural and bulky genera *Cereus* and *Pilocereus*. The authors divide CEREANAE into 38 smaller and more natural genera. In this group are the giants of the family, the huge *Cereus peruvianus* and its allies; the strange genus *Cephalocereus*, of which *C. senilis*, covered with long curled white hairs, is popularly known in Mexico as the "old-man cactus"; the gigantic *Pachycereus Pringlei* from Sonora and Lower California, which reaches 11 m. in height; the even taller *Pachycereus chrysomallus* and *P. columna-trajani* from Puebla and Oaxaca, Mexico; and the giant cactus of Arizona and southeastern California, the *Carnegia gigantea*.

The subtribe HYLOCEREANAE includes all the vinelike species with trailing, climbing, or pendent branches, formerly in *Cereus*. They invariably emit aerial roots on the sides of their joints. Some of them are rampant high climbers, and many of them produce the finest and largest flowers among plants. They fall into nine very natural genera, of which *Hylocereus* and *Selenicereus* are both night-blooming and have the most showy and very fragrant flowers. *S. grandiflorus* is popularly known as "queen-of-the-night."

Volume III deals with the subtribes ECHINOCEREANAE, ECHINOCACTANAE, and CACTANAE, which contain most of the smaller forms, many of which are one-jointed. The first subtribe includes the North American genus *Echinocereus*, which extends from Mexico into the western United States, and as far north as Wyoming and Utah. They are all low plants, simple or branched at base, mostly with beautiful flowers, which easily grow and flower in cultivation as pot plants. BRITTON and ROSE describe several new species, so that the genus now numbers 60 species, whereas SCHUMANN had only 38 species. The other genera of this subtribe are all South American, usually with globular bodies, often growing in clumps, and in many cases having beautiful and highly colored flowers. They were included by former authors under various incongruous genera, while the new genera here proposed are most natural and concise. Some of these genera, particularly *Echinopsis*, have been known in cultivation, while the little red-flowered *Chamaecereus silvestri* and the little round-bodied *Rebutia minuscula* are recent introductions from Argentina.

The subtribe ECHINOCACTANAE is divided into 28 small genera, mostly made at the expense of the old heterogeneous genus *Echinocactus*. SALM-DYCK was dissatisfied with this genus, but despite several attempts made later to modify it, SCHUMANN still retained it. It is impossible in a short review to enter further into the details of these 28 genera. Suffice it to say that they seem well founded, although I would have divided *Ariocarpus* into two, and I regret that the rules of nomenclature obliged the authors to employ such an ugly sesquipedalian name as *Echinofossulocactus*, for which SCHUMANN's subgeneric name *Stenocactus* would be preferable if allowable. Some of the giants of this subtribe are retained under *Echinocactus* as established by LINK and OTTO in 1827; here belong *E. grandis*, *E. ingens*, and *E. visnaga*, 50–100 cm. in diameter, and often 1–3 m. high.

The dwarfs of this subtribe are *Epithelantha micromeris* from western Texas and northern Mexico, and the eight species of *Frailea*, all natives of South America, some of them only 1 cm. in diameter. Of great biological interest is *Lophophora Williamsii*, which during its resting period sinks almost entirely into the ground and hides itself under the dust until the rainy season sets in, swelling it up and washing it clean, so that it reappears above ground and opens its small pink flowers. It is devoid of spines. Of great interest also are the representatives of *Ariocarpus*, with their horny and in some species fissured epidermis, which makes them look much like the surrounding stones where they grow, and which often nearly cover them. *A. fissuratus* is said to be easily overlooked among the rocks when not in flower. Some of the species are often called rock cactus. This is a case of parallelism shown in the mimicry of a number of species of *Mesembrianthemum* in South Africa.

Of the subtribe CACTACEAE, *Cactus*, or *Melocactus* as it was commonly called by former authors, is popularly known as "Turk's cap," alluding to the cylindrical flower and fruit bearing cephalium on the top of the plant body. These species, which are very common in the West Indies, are extremely variable, and an almost incredible number of so-called species had been described, but happily the authors, after a careful study in the field, chiefly by BRITTON, were able to reduce the number of real species to 18. They range from Brazil to the West Indies on the east, and from Peru through Central America to southern Mexico on the west.

Volume IV concludes the tribe CEREAE with the last three subtribes. The subtribe CORYPHANTHANAE contains 14 genera, and is built up partly from the *Echinocactus* and *Mammillaria* of older authors. These plants, with a few exceptions, have globular bodies, and small but brightly colored flowers, and many of them are favorites among amateur cactus growers. Unfortunately, because the old familiar name *Mammillaria* is a homonym of a genus of Algae, it had to be changed. It is named *Neomammillaria*. Despite the fact that a large number of species were taken out of the old genus *Mammillaria*, of which SCHUMANN knew 105, so many new species have been added that in this much more restricted sense BRITTON and ROSE recognize 150, the bulk of which (122) are native of Mexico.

The subtribe EPIPHYLLANAE contains mostly epiphytic plants, often with flat, leaflike, and spineless joints, and some with very showy flowers, of which *Zygocactus* and *Epiphyllum* (the *Phyllocactus* of SCHUMANN and other authors) are much grown stove plants. The critical treatment of this subtribe is especially interesting, and many new facts are recorded.

The eighth and last subtribe, RHIPSALIDANAE, is divided into 8 genera mostly new or revived. They are almost all graceful epiphytes, with small and usually white flowers. The student of the very difficult genus *Rhipsalis* will be glad to see almost every species illustrated.

An extended appendix enumerates a great amount of additional data, including a number of new species, and even two new genera, which were discovered while the volumes were passing through the press. A very careful and complete index covers 28 pages, with four columns to the page, and contains about 10,000 plant names.

Except for ENGELMANN'S classical publications on the Cactaceae, no comprehensive study of this family, almost exclusively American in its origin, had come from America. The Carnegie Institution and the authors deserve great credit and the thanks of botanists for presenting such a full and attractive account of these remarkable plants.—A. BERGER.

Age and area

Among the theories propounded during the past two decades to account for the facts of plant distribution, none has attracted more attention than the "age and area" hypothesis of WILLIS. Formulated in 1915, it has been elaborated in a series of publications appearing from time to time as the author's studies have progressed and new data have been accumulated. This journal has noted the gradual development of the theory,[2] and now welcomes a volume in which the substance of previous articles is brought together in organized and concise form.[3]

WILLIS states the theory as: "The area occupied at any given time, in any given country, by any group of allied species at least ten in number, depends chiefly, so long as conditions remain reasonably constant, upon the ages of the species of that group in that country, but may be enormously modified by the presence of barriers such as seas, rivers, mountains, changes of climates from one region to the next, or other ecological boundaries, and the like, also by the action of man, and by other causes."

The present position of the theory is discussed in the first part of the book, particularly in a chapter contributed by GUPPY. Among the points emphasized as fundamental are the origin of each species in one limited area, and its migration from the small area to a larger; the relative slowness of this migration and the general similarity of the results under somewhat similar conditions; the rapid spread of introduced species usually due to the changes of conditions that have been made by man; and the fact that acclimatization under natural conditions extends over vast periods of time with very small steps leading to vastly different conditions.

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[2] Rev. in BOT. GAZ. 61:82–83. 1916; 62:160–161. 1916; 63:419–420. 1917; 65:116–117. 1918; 65:486. 1918; 70:324–325. 1920.

[3] WILLIS, J. C., Age and area: a study in geographical distribution and origin of species. 8vo. pp. vii+259. London: Cambridge Univ. Press. 1922.

Recognizing these conditions, it is shown that predictions based on the hypothesis have been verified. It is also evident that most of the objections that have been urged against the theory are due to misunderstandings of its terms or to the neglect of some of its qualifications. One of the most common errors has been to show that it fails when applied to one or two species, while the author distinctly states that it is to be applied to groups of ten or more.

The second part of the volume opens with an extension of the main hypothesis under the name "size and space." This may be expressed as "on the whole, keeping within the same circle of affinity, a group of large genera will occupy more space than a group of small. The space occupied will vary more or less with the number of species." In a chapter by SMALL it is shown that in the Compositae the average generic area and the average number of species per genus vary together, and are closely related to absolute age. Mrs. REID contributes a chapter in which paleobotanical evidence is advanced which is favorable to the age and area hypothesis, and in another DEVRIES shows that it harmonizes perfectly with and indeed depends upon the origin of species by mutation.

Other topics as indicated by chapter headings are endemism and distribution, monotypic genera and genera of larger size, the hollow curve of distribution, the applicability of age and area to animals, and the origin of species.

In a more recent paper[4] are replies to certain criticisms that have been made, the principal ones being based on the failure of the critics to realize that the theory cannot be applied to less than ten allied species at once, and the view that endemic forms are chiefly relics. In reply WILLIS asserts that although relic endemics are common, they are quite lost in the crowd of initials. It is shown that the nearer an island is to the mainland, the larger the proportion of monotypes among its endemics, a phenomena which is difficult to explain if endemics are mostly relics. In spite of the logical nature of these replies, it seems to the reviewer that too little weight has been given to relic endemism, and insufficient account taken of the dying out of species. This, however, would not invalidate the theory as a whole, nor prevent its application to most regions of the world. WILLIS is certainly to be commended for the vast quantity of data upon which his hypothesis has been founded, and for its stimulating effects upon studies of geographical distribution.—GEO. D. FULLER.

[4] WILLIS, J. C., Age and area: a reply to criticism with further evidence. Ann. Botany 37:193–216. 1923.

The pharmacists' botany

Botany is a many sided science, and many textbooks have been written attempting to meet the need of students of plant life. One group of students, however, has been neglected hitherto, but their needs are now met by RIGG,[5] who has prepared a textbook of botany for the students of pharmacy. The book reminds us that once botany was mainly a study of drug plants, and that physicians were the chief botanists.

The book is very readable, but quite elementary, testifying to the grade of requirements demanded of pharmacists so far as botany is concerned. It might very well be used with high school students, except for the scientific nomenclature of drug plants. From much of the text one feels that after all what is wanted is a knowledge of the same facts about plants that the student obtains from any other good elementary textbook of botany, but it gets its pharmaceutical flavor by having the examples and illustrations taken from the list of drug plants in the U.S.P., and by a very brief paragraph at the end of each chapter, bearing some such title as "some pharmaceutical buds," "some pharmaceutical leaves," etc., so that the student will know that he is really getting something that will help him in pharmacy.

The text is organized into three parts. Part I is devoted to seed plants, with chapters on organs, the cell, plant names, leaves, stems, buds, roots, physiological processes, flowers, seeds, fruits, life histories, and the classification of seed plants. The last of these chapters is unique in consisting mainly of a list of about thirty-four pages of official and unofficial drugs from the various families of seed plants. Part II deals with spore plants, and contains chapters on thallophytes, bryophytes, and pteridophytes. Part III, on some other phases of pharmacy botany, considers ecology, propagation, and breeding as subjects contributory to the growing of drug plants.

The appendices present an outline of the plant kingdom, and a set of botanical synonyms of the common names used. An unusually full index enables one to locate all the references to each plant mentioned in the text. As the author has had fourteen years of successful experience in teaching botany to pharmacy students, the book should commend itself to others who are engaged in similar work. Students will find it entertaining and instructive, and the text surely does not demand too much of them.—C. A. SHULL.

MINOR NOTICES

Vegetation maps.—The lack of adequate and accurate maps of the vegetation of the world has been appreciated and deplored by all ecologists and plant geographers. These workers will therefore welcome certain recent additions as indicative of actual progress. GOODE'S[6] *School Atlas* contains a number of such maps, including one of the vegetation of the world after SCHIMPER. It is a decided improvement on the original, and if the coastal strip of rain forest in eastern Africa could be transferred to the corresponding coast of South America, it might be regarded as quite satisfactory. In the same publication is a map of the vegetation of the United States and Canada. The plant covering of the former country is accurately and carefully delimited by SHANTZ and ZON of the United States Department of Agriculture, but the utility of the entire map is almost destroyed by the errors in the Canadian portion. A long strip of forest characterized by chestnut and chestnut-oak extends from Toronto and along both sides of the St. Lawrence River to the Gaspé peninsula. This is very misleading, for there is certainly not a tree of either species to be found in the Province of Quebec. Then the coincidence between the boundary line which separates Maine and New Brunswick and that which divides two forest formations is as absurd as it is erroneous. The two countries are parts of one physiographic and phytogeographic unit, artificially separated by an international boundary line determined by political compromise.

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[5] RIGG, G. B., The pharmacists' botany. 8vo. pp. xvii+303. New York: Macmillan Co. 1924.

[6] GOODE, J. P., School atlas; physical, political, and economic. pp. xii+41. 96 plates of maps. Chicago and New York: Rand McNally and Co., 1923.

Perhaps the most important of the recent phytogeographic maps is one of Africa, prepared by SHANTZ[7] for the use of the "American Commission to Negotiate Peace." The wealth of material reviewed and digested in producing this map and the accompanying descriptive text is indicated by a bibliography of 400 citations. The vegetation is classified along ecological lines into twenty-one types or formations, among which tropical rain forest, various savannas, and deserts are most conspicuous. These formations are represented on the map in a manner that results in easy legibility. Accompanying the vegetation map are others of rainfall distribution, soil, and land classification.

A minor contribution has been made to our knowledge of the vegetation of South America in a map of Peru by WEBERBAUER,[8] on which the distribution of twenty-five association types is delimited. The accompanying text has brief descriptions of these types.—GEO D. FULLER.

Determination of hydrogen ions.—The second edition of this excellent treatise[9] reflects in part the rapid advances made in hydrogen ion concentration studies during the several years since the appearance of the first edition. The book has been revised where necessary, and some slight changes of organization have been made. Thus the first and eleventh chapters have each been divided into two, increasing the total number of chapters to twenty-one. The chapter on the choice of indicators has been much enlarged, and the chapters dealing with approximate determinations of hydrogen ion concentration, hydrogen electrodes, and calomel electrodes are somewhat more extensive. Two tables have been added to the appendix, giving the standard values for calomel electrodes, and the relation of H+ to P_H. The literature citations occupy about forty pages in this edition.

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[7] SHANTZ, H. L., and MARBUT, C. F., The vegetation and soils of Africa. pp. 263. *pls. 2. figs. 50.* New York: Amer. Geogr. Soc. Research Series no. 13. 1923. $5.00.

[8] WEBERBAUER, AUGUST, Die Vegetationskarte der peruanschen Anden zwischen 5° und 17° S. Petermanns Geogr. Mitteil. **68**:89–91, 120–122. map. 1922.

[9] CLARK, W. M., The determination of hydrogen ions. 8vo. pp. 480. Baltimore: Williams & Wilkins. 1923.

The book is well made, and the author and publishers are making a valuable contribution to the advancement of research by keeping this invaluable manual up-to-date.—C. A. SHULL.

NOTES FOR STUDENTS

The primitive spindle.—BOWER[10] has published his conception of the significance of the "primitive spindle" in the embryology of the plant kingdom, unifying all the groups, from algae to seed plants. According to this view, the first cleavage of the zygote defines the polarity of the embryo, always being at right angles to the axis connecting the two poles. This cleavage results in "a spindle-like structure, and the term primitive spindle may be applied to it." It is stated that "comparison of plants in the embryonic state shows that a filamentous or spindle-like structure, with polarity defined by the very first embryonic cleavage, is common for them all." The relations to the primitive spindle of the various organs of the different groups are given in detail. For example, "the first root of all Pteridophytes that have a suspensor is clearly lateral. It projects from the side of the primitive spindle, and is not itself a part of it." In this way the different organs are interpreted, and the embryology throughout the whole plant kingdom referred to one fundamental organization.—J. M. C.

Fertilization in cotton.—KEARNEY[11] has investigated the problem of crossing and selfing in cotton, especially with the Pima variety of Egyptian cotton, although comparisons with upland cotton are included. The experiments were conducted at Sacaton, at the Pima Indian Agency in southern Arizona, and extended through eight years. He finds that, although the cotton flower is well adapted to cross pollination, most of the ovules are usually self-fertilized, the percentage of hybrids produced when two distinct varieties are grown side by side being small. He also investigated the structure and later ontogeny of the flower, the deposition of self pollen and foreign pollen upon the stigmas, and the competition of the two kinds of pollen. The bulletin is an interesting addition to the literature of pollination.—J. M. C.

[10] BOWER, F. O., The primitive spindle as a fundamental feature in the embryology of plants. Proc. Roy. Soc. Edinburgh 43:1–36. *figs. 26.* 1922.

[11] KEARNEY, T. H., Self-fertilization and cross-fertilization in Pima cotton. U.S. Dept. Agric. Bull. 1134. pp. 68. 1923.

GENERAL INDEX

Classified entries will be found under Contributors and Reviewers. New names and names of new genera, species, and varieties are printed in **bold-face** type; synonyms in *italic.*

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